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NASA CONTRACTOR REPORT

NASA CR-661

NASA CR-661

FACILITY FORM 602

N67-24421 (ACCESSION NUMBER)

122 (PAGES)

(NASA CR OR TMX OR AD NUMBER)

(THRU)

(CODE)

04 (CATEGORY)

EFFECT OF DIET AND ATMOSPHERE ON INTESTINAL AND SKIN FLORA

VOLUME I - EXPERIMENTAL DATA

by Lorraine S. Gall and Phyllis E. Riely

Prepared by
FAIRCHILD HILLER CORPORATION
Farmingdale, Long Island, N. Y.
for Manned Spacecraft Center

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • APRIL 1967

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By Lorraine S. Gall and Phyllis E. Riely

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Prepared under Contract No. NAS 9-4172 by
FAIRCHILD HILLER CORPORATION
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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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SUMMARY

Eight young male subjects were confined for a period of 34 days, two in a control area and six in a chamber that was at altitude with 100% oxygen for 20 days. Minimal hygiene procedures with and without space suits and space-type diets were other experimental variables.

Aerobic and anaerobic microbiological studies were carried out at frequent intervals on several body areas, including axilla, groin, glans penis, throat and buccal areas and the feces of eight subjects and their environment, during an experiment to test the effect of minimal hygiene procedures in a confined area, space diets, 100% oxygen at 5 psia and the wearing of space suits on these men. Several conclusions can be drawn from this work.

1. The types of microorganisms found in each of the body areas and feces were in good agreement with those regarded in published reports and in other Republic Aviation Corporation studies as normal body microflora for that area of the body. The kinds of microorganisms found in the environment reflected the hardier types of body microorganisms isolated from the subjects.

2. The total number of colonies enumerated on aerobic blood plates in all body areas and in the environment increased as the experiment progressed. In general, the buildup in the axilla, groin, G. P. and buccal area reached a plateau by the mid-point of the experiment and then stayed relatively constant or decreased, while the buildup in the throat flora was more variable. The increase in bacteria in the chamber area was larger and fluctuated more than in the cottage.

3. The bacteria involved in the buildup of microflora in the axilla, groin and G. P. were staphylococci or micrococci and corynebacteria, with the corynebacteria predominating in the groin and G. P. of the subjects and in the axilla of four of the subjects in the last sampling periods. Staphylococci predominated in the axilla of the other four subjects. Streptococci were involved in the increase in microorganisms in the throat and buccal area. The bacteria involved in the buildup in the environment were largely staphylococci or micrococci, gram negative rods and, to a lesser extent, streptococci.

4. The buildup of bacteria on the body areas occurred in such a pattern that it appears to be the result of the minimal personal hygiene procedures with the subjects in a confined area and the increase in bacteria was of such a nature that it does not appear to be harmful to the subjects for a period of 34 days. The other experimental conditions in this trial did not seem to influence the body or environmental microflora.

5. The potentially pathogenic bacteria, Shigella Poly B, Bethesda-Ballerup and coagulase-positive phage-typable staphylococci were isolated from certain subjects during the experiment; but these bacteria did not cause overt illness and did not appear to transfer readily from one subject to another.

7. The types and numbers of bacteria found in the wash water from the "space sink" contributed to the abandonment of the use of these sinks, and the wash cloths used in the latter part of the experiment were unsatisfactory also when used continuously. The methods of urine collection, and cleaning the space suits prior to donning, were not satisfactory from a microbiological standpoint.

6. In the feces strict anaerobes represented over 95 percent of the predominating bacteria and outnumbered the aerobes by more than 1000:1. In general, the types of fecal anaerobes isolated, as well as the frequency of their occurrence, agreed well with the distribution of the bacteria described as FA types on the basic NASA study, with one significant exception. After the subjects had been on the experimental diet for about two weeks, the type of fecal anaerobes designated as GD started to increase and continued to be isolated frequently for the remainder of the trial. This change in fecal anaerobes probably is diet connected, as it was similar to the shift noted on another experiment in which diet was the main variable.

Recommendations for consideration in future studies include a thorough microbiological study to be done on future subjects prior to the experiment to detect carriers of potentially pathogenic bacteria; better methods of cleaning the face and hands, collecting urine, and cleaning the suits to prevent microbiological contamination; and "total" bacterial counts of the body areas made from two sets of blood agar plates, one incubated aerobically and the other under CO_2.

INTRODUCTION

The health and welfare of the astronaut is of prime importance during space flight so that he is capable of rendering peak performance at all times during his mission. One factor that can impair the health of the astronaut is related to the microbial flora which live in or on his body or which are present in his environment. For this reason it is necessary to study the effect of conditions of space flight upon these indigenous microorganisms, since knowledge of the alterations in the occurrence and metabolism of individual microorganisms, or in the balances of organisms under these conditions is necessary in maintaining the health and welfare of these astronauts.

Certain conditions of space flight such as personal hygiene procedures, wearing of space suits, atmospheric composition and pressure, confinement and a space-type diet are particularly apt to influence the microbial flora of the astronaut. For example, the type and frequency of personal hygiene procedures such as bathing, brushing of teeth, shaving and toilet routines play an important role in the establishment of the resident flora of the exposed areas of the human body. A requirement of space travel is the minimal use of personal hygiene procedures, which often are limited to the use of face cloths without a cleansing agent and brushing the teeth without a dentrifice. The effect of the less complete hygiene methods on the resident microflora of men living under these conditions is important. Microbial studies underway at Wright-Patterson under contract AF33(615)-1814, Biomedical Criteria for Personal Hygiene[1], where men have been confined to a chamber under ambient conditions for four weeks and have used minimal personal hygiene procedures indicate that certain bacterial populations tend to buildup in some body areas, particularly in areas of heavy perspiration; but that no serious health problems have developed in these men. However, potentially pathogenic bacteria have been isolated, and further study superimposing certain space stresses are needed to evaluate this finding as related to space flight.

The wearing of a closely fitting space suit, with air or oxygen circulating through it for prolonged periods, will create an altered environment for the microorganisms on the skin of the astronaut. Problems may arise both with

respect to possible areas of contact irritation and to the effects of humidity. The close-fitting suit may rub a certain body area, creating an abrasion ideal for microbial infection. Areas of the body where perspiration is normally heavy may remain damp, while areas near the air intake may be abnormally dry. The variable humidity plus the altered atmospheric flow may well influence the bacterial population. In fact the high oxygen atmosphere itself coupled with reduced atmospheric pressure may also influence the character of the bacterial flora.

The close confinement imposed by space flight will cause considerable interaction between the microbial flora of the several astronauts with each other and with their environment. The spread of potentially pathogenic microorganisms from a "well" carrier to another astronaut directly or by way of the common environment presents a problem and microbiological studies conducted on men in simulated space chamber experiments under constracts NASr-92[2] and AF33(615)-1814[1] have shown the probable transfer of pathogenic bacteria from one subject to the others. The mode and frequency of such transfer of potential pathogens is of importance in maintaining the flight crew efficiency.

Diet is known to influence the intestinal flora of animals and recent studies conducted under Wright-Patterson contract AF33(615)-1748[3] at Republic Aviation Corporation have shown that a space-type diet affects the human fecal flora, especially with respect to the increased occurrence of a group of gas-forming, proteolytic anaerobes. Since flatulence and odors from intestinal gas may be a problem in space flight, the influence of diet on the intestinal flora must be clarified.

From this discussion it is evident that several questions remain to be answered relating to the microbiological aspects of space flight. Specifically, does the bacterial population of the astronaut or his environment buildup during space flight and if so, with respect to which types of organisms? Do any of the factors of space flight favor this buildup? Is this buildup harmful to the health and well-being of the astronauts? Do any of the pathogenic organisms present a problem during space flight? Are the astronauts healthy carriers of potentially pathogenic organisms and do they transfer these organisms to fellow crew members? If so, does this create a health hazard? If any of the foregoing considerations create problems, what can be done to alleviate them? Only a comprehensive microgiological study on humans under simulated space conditions can offer the answers to these questions.

For these reasons an extensive study was done on the types and numbers of microorganisms present and the frequency of their occurrences in six body areas of six young men subjected to certain conditions of space flight and their two controls for a 34 day period in a recent study in the ACEL chamber at the Pennsylvania Navy Yard. Under contract NAS-9-4172, samples for microbiological culturing were taken at the start of the trial and at frequent intervals during the test, and the kinds and numbers of bacteria isolated in each sampling period were studied. The bacteria present in their environment were also determined in the same manner.

The experiment was designed in such a way that the six experimental subjects and their controls used the same minimal personal hygiene procedures and ate the same dehydrated foods throughout the entire experiment. Both the test subjects and their controls lived in confined areas which in the case of the controls was maintained at ambient conditions. This was in contrast to the test subjects who were maintained at an altitude of 27,000 feet under 100% oxygen for a total of three weeks and wore space suits during the last two weeks at altitude and for one week post-altitude. The control subjects wore space suits during the last three weeks of the trial.

Since the microbiological determinations were carried out throughout the entire experimental period, the effect of the various experimental conditions can be evaluated in terms of their influence on the various members of the microbial population.

The scope of work can probably be best appreciated by quoting several figures: 1378 samples were taken from the body areas and the environment as well as from the urine bottles, wash water and the suits, which resulted in 18,500 odd primary cultures. To study these cultures, over 150,000 plates and tubes of media were used in secondary culturing and over 10,000 slides were made and observed. The results of these studies have been summarized in table form at the end of this report and present the total numbers and occurrences of aerobic and anaerobic bacteria found on the various body areas tested and in the environmental areas of the chamber and cottage.

METHODS

Eight young men from the United States Armed Forces were subjects in a chamber trial to determine the effect of a pure oxygen atmosphere at altitude combined with the wearing of space suits, minimal personal hygiene procedures, and eating a space-type diet upon their health and well-being. Six men were confined to the ACEL chamber for a period of 34 days and two men served as controls in a nearby installation termed the "cottage". The exact experimental conditions have been defined elsewhere, but in brief the subjects in the space chamber were under ambient conditions for one week, at altitude with no space suits for one week and with space suits for two weeks, after which the chamber was maintained under ambient conditions, but the subjects wore the space suits. The men in the cottage were under ambient conditions at all times but wore space suits for the last three weeks of the trial. All eight men ate the dehydrated food and used the minimum personal hygiene procedures of washing only the face and hands with a face cloth with no cleanser and brushing the teeth with no dentrifice for the entire experimental trial.

The microbiological samples were taken according to the schedule indicated in Table 1. All eight men were scrubbed thoroughly, as were the chamber and cottage, prior to the first sampling. The exact details of the collection and processing of the samples are contained in Appendix A, but in brief two swabs - one for aerobic and one for anaerobic culturing - were taken from the following body areas: throat, buccal area, axilla, groin, glans penis, and eye (first sampling only) for a total of 10 samples from each area and fecal samples were obtained approximately twice a week as indicated in Table 2. Fourteen samples were taken from each of several environmental areas in both the chamber and the cottage as indicated in Table 1 by means of sedimentation plates or swabs. In addition cultures were made from the urine collection bottles, water squeezed from face cloths and from certain areas of the suit prior to donning and from the suit vents after donning at stated intervals indicated in Table 1.

The detailed anaerobic and aerobic experimental procedures for obtaining each sample and the technique and media used for the primary culturing for each of the body areas are included in Appendix A. The culturing of the samples from

the body areas was done at the site of the ACEL chamber and the culturing was done immediately following the collection of the samples by the subjects. The fecal samples were cultured immediately following elimination on an individual basis. After proper incubation the primary cultures on solid media were then transferred to Republic Aviation Corporation laboratories for processing according to the schema set forth in Appendix A. Selected broth cultures from the anaerobic series were transferred into agar shakes at the primary culturing site for transport to Republic Aviation Corporation's laboratories. The cultures that were made from the environmental areas and from the miscellaneous items were treated in the same manner as the cultures from the body areas.

Slides were made from all aerobic and anaerobic cultures showing growth and slides were also made of all original samples at the time of primary culturing. None of the samples collected appeared to be abnormal in character.

The data from both the aerobic and anaerobic culturing (when done) for each body area sampled, from the feces, the environmental areas, and the miscellaneous items are recorded in tabular form and are considered both with respect to the microflora of each subject or environmental area and with respect to each sampling period which reflects the effect of the various test conditions.

The data recorded in the tables refers to the subjects by number as shown in the following list:

Subject Number	Name	Area
1	Yung	Chamber
2	Strickler	Chamber
3	Abbitt	Chamber
4	Foster	Chamber
5	McBride	Chamber
6	Munger	Chamber
7	Juergens	Cottage
8	Pipkin	Cottage

EXPERIMENTAL RESULTS

The results of the study of the indigenous microflora of various body areas of the eight young male subjects in this experimental trial are summarized in detail in the tables. A brief work description of these results will be presented in this section in the following order:

(1) Total numbers of bacteria found in the various body areas tested, excluding the feces, and from the environmental areas of the chamber and cottage

(2) The occurrence of the various types of aerobic bacteria from the body areas and feces and the environmental areas of the chamber and cottage

(3) The occurrence of the obligate anaerobic bacteria in the feces and body areas

(4) The buildup of specific types of bacteria in the body areas

Total Aerobic Counts

The total numbers of bacteria present in each body area tested in each sampling period for each subject was estimated by total bacterial colony count taken from blood plates incubated aerobically for 24 hours, and the results are summarized for each body area separately according to subject and period in Table 3 and summarized for all body areas for each subject in each sampling period in Table 4 . In general, directly after the preliminary showering, the total bacterial count from the axilla for all but one subject was quite low, but increased markedly in the second and third sampling periods in six of the eight subjects so that the plates had too many colonies to count. Accordingly, for all body areas the dilution of the sample before plating was increased so that the colonies on the plate could be more easily counted, and in the fourth sampling period the plate from all but one subject could be counted. The two controls in general showed high counts for the remainder of the trial, while chamber Subjects 2 through 6 usually exhibited somewhat lower counts than

the two men in the cottage. Subject 4, and usually Subject 2, showed a low count for the remainder of the test. Subjects 3 and 6 maintained relatively high counts throughout the entire series, while Subjects 1 and 5 showed alternating low and high counts. The overall pattern seemed to indicate a gradual buildup of organisms in the axilla on all subjects with the possible exception of Subject 4, but the buildup seemed to be more consistent in the subjects in the cottage.

The total bacterial plate counts from the groin were also low for all subjects following the shower and, as was the case with the axilla, in the second sampling period and in most instances on the third sampling period there were too many colonies on the plate to count and in all but one instance the total count had increased over the original count. Also as with the axilla, both subjects in the cottage showed more consistently elevated counts with the exception of one or two sampling periods (6 and 7) than the subjects in the chamber. All of the subjects in the chamber showed an increase in total bacterial count in the groin during almost all sampling periods, especially during the last two or three sampling periods. Subject 1 showed the least buildup of organisms in the groin until the final sampling period when the count increased suddenly. Thus it did appear that the subjects in the cottage showed the most consistently high total bacterial counts from the groin and the other subjects showed a variable buildup in total bacterial counts which in general were lower than the average in the cottage.

The low initial total bacterial plate count from the G. P. showed that in general the washing of that area during the shower was effective in reducing bacteria. Six out of the eight subjects showed a marked increase in bacterial counts during the second and third sampling periods, the exceptions being Subjects 5 and 7, both of whom had started with rather high counts in the first sampling period. In general all the subjects showed a marked increase in the total bacterial counts of the G. P. throughout the trial. Some variations were noted in individual sampling periods but by the fourth sampling period there was a marked overall increase in the numbers of organisms cultured. Subject 4 showed perhaps the least marked buildup. There appeared to be no great

difference between the buildup of total bacterial counts on the subjects in the chamber or in the cottage.

The total bacterial plate count in the buccal area showed marked individual variation from period to period with very little apparent correlation with the sampling period. There was a marked overall increase in the organisms isolated from the buccal cavity as the trial progressed, starting with the fourth or fifth sampling period, which was particularly striking in the subjects in the chamber.

Total bacterial plate counts from the throat were rather moderate at the start of the trial with the exception of Subject 1 and no overall general increase was noted until about the sixth or seventh sampling period. The increase became more marked in the eighth sampling period. This increase was maintained in four subjects during the ninth period after which all but one subject, Subject 1, showed a marked decrease in the counts. With respect to the total colony counts from the throat, the fluctuations were correlated with the individual, but the overall count built up as the trial progressed.

The aerobic total plate counts were often rather erratic from period to period, particularly with respect to the axilla, groin and G. P. areas. This may be partially explained by the fact that in the periods when there were lower aerobic plate counts frequently the blood plate incubated under CO_2 showed high counts usually made up largely of very tiny colonies. This might suggest that the organisms growing on the CO_2 plate were not able to grow well on the aerobic plates and thus the "total" count was deficient by the number of bacteria encouraged to grow by CO_2.

This observation was strengthened by the results of comparing the height of growth in the aerobic vs anaerobic broth dilution series from five body areas, which is summarized for each subject for each period in Table 5 and an overall average is found in Table 6. This broth series showed consistently that the anaerobic series grew to a higher dilution than the aerobic for all body areas. The overall average showed about half a dilution or five times difference between the aerobic and anaerobic growth in the broth series.

It is difficult to make a direct comparison between the numbers of bacteria as estimated from the total plate counts and the broth dilution series. because the dilution represented by each tube in the broth series was quite large, as is shown in Table 7 , but is usually in the order of 1:40 after the fourth period. Thus accurate counts could not be determined using the broth series. However, in the broth series, in general, the height of growth in period one was lower than the height of growth from the same body area in periods two and three. In order to ensure enough tubes in the series to allow maximum growth the dilution factor was raised in period four and again in period five, after which it remained constant for the rest of the experiment. Taking these changes of dilution into account, the data from the broth series also showed that there was a buildup in the total organisms for each of the body areas. Thus both from the aerobic plate counts and the growth in the broth dilution series, it would appear that there was a buildup of total bacteria in each of the body areas as the experiment progressed.

The average total colony counts from the chamber and cottage subjects considered separately by period are recorded in Table 8. These data show that the bacterial counts increased in all body areas as the experiment progressed, and that the subjects in the cottage showed a greater overall buildup in the axilla and the groin than those in the chamber, while the reverse was true in the buccal area.

Exposure plates were used to gather total counts in four areas of the chamber and cottage and the results are recorded in Table 9. The initial samples in the chamber showed that the cleaning had been very effective and beginning with the second sample increases in bacterial counts were found in all areas. There appeared to be a somewhat consistent buildup during the next six sampling periods, reaching a climax in the sixth sampling period when three out of the four areas showed bacterial populations too numerous to count. Beginning with the seventh sampling period there was a marked decrease in counts followed by a marked increase in the eighth period. The ninth period showed a marked decrease again, which was maintained during the tenth sampling period. An increase was noted in the bacterial count in

in three out of four areas tested during the eleventh period and all four areas showed high counts during the twelfth period. These counts became lower during the thirteenth and fourteenth periods. These rhymthic fluctuations cannot be explained on the basis of filter changes or other physical happening, but removal of the filter after period eight may account for the low counts in period nine.

The total bacterial counts for the cottage showed quite a different pattern. The personal hygiene and table areas showed 54 colonies each during the first sampling period whereas the bed and TV showed no organisms initially. However, by the second sampling period and for the remainder of the experiment the table, bed and TV showed either no organisms at all or fewer than 50 in any one sampling period. The personal hygiene areas increased immediately in the second sampling period to 150 and sporadically showed counts in excess of 50 throughout the remainder of the test period. Thus, whereas the chamber showed periodic buildup and declines in total bacterial counts, three areas in the cottage remained relatively stable in total bacterial population and the personal hygiene area in the cottage showed higher but fluctuating total bacterial counts.

At five intervals during the test period, the wash water was examined for the total organisms present in 1 ml of the wash water. These counts were very revealing as is shown in Table 10. During the first period of the experiment two subjects each shared a "space sink". After one week of use, wash water drawn from these space sinks had too high a bacterial concentration to be counted on the plates (See Figure 1). Accordingly, the use of the space sink was abandoned and each subject was given his own wash cloth. After a week of use the water squeezed from this wash cloth showed relatively low bacterial counts (about 200 to 600). However, after this wash cloth had been used for another week the counts on all but one subject, 5, were either too numerous to count or exceeded 1000 bacteria per ml. These wash cloths were discarded and new wash clothes were supplied. After these cloths had been used for a week they were sampled and the counts varied in most cases from about 500 to 1500 with one subject, 2, having a heavy bacterial population. These same

cloths were used for another week after which they were tested and all but one subject, 7, showed extremely heavy bacterial populations. Since the organisms isolated were mainly coliforms or staphylococci these findings seemed to be quite unsatisfactory from the standpoint of personal hygiene.

The urine bottles were cultured several times during the experiment to determine the accumulation of organisms around the neck of the bottle. The results from all three sampling periods were similar and the results from the April 19 sampling are shown in Table 11. In all subjects except Subject 7 the counts were in excess of 500,000 and in four instances were too numerous to count. The organisms found in this examination were largely coliform organisms. Since the neck of the urine bottle was exposed to the general environment, the use of such urine bottles seems to be an undesirable practice.

Prior to donning, each suit was sampled by a swab which was plated for the presence of bacteria in the axilla, crotch and right foot. The results are recorded in Table 12. This examination revealed that organisms were found in at least two areas in all but suits 3 and 4. Three suits had staphylococcus organisms in excess of 200,000 per swab and five suits showed the same type of organism in amounts of 100,000 to 10,000,000. The right foot of four suits showed staphylococci in numbers varying from 400,000, 2,000,000 to 500,000. These results would indicate that considerable amounts of organisms were carried over from the previous wearing of these suits.

The total bacterial counts on the blood plates exposed to the vents on the suits were in general quite low and it did not appear that under the circumstances of this experiment undue numbers of bacteria were being expelled through the vent.

Gram Positive Cocci

The results of the total bacterial counts from the various body areas show that there was a buildup of total organisms. The following discussion presents the data on the types of organisms present.

The gram positive cocci were the most numerous types of bacteria isolated from several of the body areas during most of the trial period. The occurrence of gram positive cocci in each subject in each period is recorded in Table 13. The staphylococci are divided into those gram positive cocci which produced acid on mannitol salt media and which subsequently proved to be coagulase negative or coagulase positive, and those staphylococcus which will grow on the mannitol salt agar without producing acid, which probably are Staphylococcus epidermidis. Many mannitol salt positive cultures occurred on Subjects 1, 2, 7 and 8 particularly on the groin, axilla and G. P. with sporadic occurrences on Subjects 3, 4, and 6. No such cultures occurred on Subject 5. It is of interest that few of these cultures were coagulase positive and these occurred mainly on Subjects 1, 2 and 7, with one isolation from Subject 4 from the groin area. The remainder were coagulase negative.

All coagulase positive cultures isolated from the subjects and the environmental areas along with four coagulase negative strains each from Subjects 4 and 7 were taken into the clinical laboratories at Roosevelt Hospital to be examined by Dr. John E. Blair, Chairman of the International Subcommittee on Phage Typing of Staphylococcus. In addition the coagulase negative strains from Subject 4 were included because he developed several pustules on his body and from Subject 7 because he so frequently had mannitol salt positive staphylococci. Dr. Blair conducted phage typing on all the strains submitted and found only two phage positive cultures isolated from the subjects, one from Subject 2 and one from Subject 7. His findings are recorded in Table 14 and will be discussed later in connection with the occurrence of phage typable staphylococci isolated from the environmental areas.

Staphylococcus epidermidis was isolated frequently from all subjects in virtually all sampling periods. This organism occurred most often on the groin, axilla and G. P. with occasional isolations from the other body areas. Micrococci were isolated frequently from all subjects in all periods, particularly from the throat and buccal areas and less frequently from the axilla than the staphylococci.

Sarcina were isolated from all but one subject, and were found in various body areas on Subjects 1, 2 and 3. It was isolated three times from the buccal area of Subject 4 and twice from Subject 5. On Subjects 5, 7 and 8 it was found only in the G. P.

The streptococci were isolated chiefly from the throat and buccal areas, although some cultures occurred on the G. P. and groin. Streptococcus mitis occurred the most frequently on all subjects in all periods followed by Streptococcus salivarius. Enterococci were found sporadically in the buccal and the throat areas of several of the subjects; but were found in these areas on Subject 3 a total of seven times, which represented nearly one-half of the total isolations of this culture. Undifferentiated Streptococcus viridans was also isolated frequently from most subjects during most sampling periods. No beta hemolytic streptococcus was found on any subject on any sampling period.

Colonies of gram positive cocci were picked from both the aerobic and CO_2 incubated blood plates and from agar shakes made from the aerobic and anaerobic broth series, and selected colonies from all sources were identified. The cultures isolated under anaerobic conditions are recorded in Table 13 in parentheses. Certain staphylococci, micrococci, sarcina and streptococci from all body areas were found to grow under the anaerobic conditions used in this experiment, so that certain types of bacteria were isolated by both aerobic and anaerobic procedures.

In summary, the specific area of the body and the individual subject seemed to influence the occurrence of the gram positive cocci more than the experimental conditions.

The isolation of the gram positive cocci from exposure plates placed in four areas of the chamber is summarized in Table 15. The same division of organisms was used in recording the results for the environmental areas as for the body areas. Mannitol salt positive, coagulase positive staphylococci were isolated from all areas of the chamber mainly beginning with the twelfth sampling period. Two of these cultures were phage typable, but were not the

same phage pattern. Also they were not of the same phage pattern as that isolated from the chamber occupant, Subject 2, who had previously shown a phage typable staphylococcus. Mannitol salt-positive, coagulase-negative staphylococci were found frequently in the various areas of the chamber from the beginning to the end of the trial. The most numerous staphylococcus isolated was Staphylococcus epidermidis which was found frequently in all areas of the chamber tested. Smaller numbers of micrococci were found sporadically throughout the test period in every area except the bed. No streptococci were found in the chamber, and only one culture of sarcina was isolated.

Similar data on the occurrence of gram positive cocci in the cottage is summarized in Table 16. Fewer isolations of mannitol salt-positive, coagulase-positive staphylococci were found in the cottage than in the chamber, but a higher proportion of these were phage typable. These occurred during the later half of the experimental trial. Two of the phage typable cultures from the cottage were the same phage pattern, but that type had not been previously isolated from the subject in the cottage. The other two phage typable strains were the same as those isolated from a cottage inhabitant, Subject 7, on a previous date. The data from the phage typing of cultures both from the subjects and their environment are presented in Table 14 according to date of isolation. Dr. Blair's comments are quoted in full. It will be noted that no typable strain was found either on a subject or his environment prior to April 1 and that no phage type occurred repeatedly. His comments are as follows:

"The large proportion of nontypable cultures among the coagulase-positive strains is not unexpected. In the absence of overt infection (a potential source of self-contamination or shedding of cocci into the environment), coagulase-positive staphylococci isolated from most body surfaces or from the environment often are less likely to be susceptible to the typing phages than are staphylococci derived from active lesions. The nose is a common site of colonization in carriers of coagulase-positive staphylococci. Studies have shown that from 40 to 60 per cent of healthy individuals may be carriers at

any given time; thus it is conceivable that at least two or three of the subjects might have been found to be carriers if the body areas cultured had included the nose.

"The eight typable cultures represented five different strains of staphylococci, as demonstrated by the five distinct phage typing patterns observed. Two typable strains were encountered in the cottage, and three in the chamber.

"That an organism harbored by a human subject can be dispersed into his environment is suggested by the strain identified by phage B5. The strain was isolated first from the axilla of Subject 7, and then from the TV and the table, respectively, 6 and 17 days later. Similarly, the strain of type 7/47/54/54 appeared on the table and on the TV in cultures taken at an interval of five days; the source of this strain (presumably human) must remain in doubt.

"The three strains isolated in the chamber are quite unrelated. One was obtained in a culture of the axilla of Subject 2, and the other two were isolated six days later from the TV and the bed. It must remain a matter of conjecture, but it would seem possible that some or all of these strains may have originated in nasal carriers among the six subjects in the chamber."

Micrococci and streptococci were isolated sporadically from the cottage areas, but there was no special pattern apparent.

Micrococci and Staphylococcus epidermidis were isolated frequently from the wash water during almost all periods tested. Micrococci occurred more often in the wash water from Subjects 6, 7 and 8, and Staphylococcus epidermidis was found chiefly in the wash water of Subjects 1 through 5. (See Table 16.)

From these data it seems that the gram positive cocci found in the environment reflect the body flora of the inhabitants, especially with respect to staphylococci and micrococci.

Gram Positive Rods

Gram positive rods comprise a significant proportion of the bacteria flora of several body areas. The occurrence of several types of gram positive rods in the five body areas and feces of the eight subjects on this trial is recorded in Table 17. Lactobacilli were found fairly frequently, principally in the feces and throat of the various subjects through the entire trial. The isolations were more numerous from certain subjects, such as Subject 1, than on others and certain subjects such as Subjects 6 and 7 carried the lactobacillus only in the feces. Bacillaceae were isolated sporadically throughout the test, principally from the groin and feces. These organisms were isolated from Subject 7 five times and from Subject 8 three times.

The most frequently isolated gram positive rods were the corynebacteria. Several species of these were identifiable, whereas others were classified merely as Corynebacterium sp. or Pattern A or X, which are described in Table 18. Corynebacterium striatum was isolated from six out of eight of the subjects, mainly from the groin and G. P. The closely related S+ variety occurred on only three subjects and again was found in the groin or G. P. Corynebacterium pseudodiptheriticum was not identified as an isolate from any subject. Corynebacterium xerosis was isolated occasionally on several of the subjects mainly from the groin or G. P., while Corynebacterium enzymicum was found sporadically on most of the subjects and represented the only identifiable corynebacterium isolated from the feces. It was also found in the other body areas, principally the groin and G. P. Corynebacterium sp. accounted for the greatest number of corynebacteria isolates and was found on most of the subjects. This type of culture occurred in all areas of the body and feces but was particularly numerous in the groin and G. P. Corynebacterium pattern A was found occasionally on most of the subjects. It occurred mainly in the groin and G. P. of several of the subjects, but occurred most frequently in the axilla of Subject 7. Pattern X occurred infrequently on most of the subjects and was found exclusively in the axilla. From these data it appears that certain types of corynebacteria are isolated most frequently from specific areas of the body,

and that body area exerts more of an influence on the types of gram positive rods isolated than the experimental condition.

It is interesting to note that although many gram positive cocci grew under anaerobic conditions, more of the staphylococci, micrococci and even certain streptococci selected for identification came from the aerobic cultures. In contrast, all of the bacilli came from aerobic cultures, whereas the corynebacteria and the lactobacilli studied originated most frequently from the CO_2 plates or anaerobic cultural series.

The gram positive rods were found so infrequently in the environment that no special table was made to discuss these results. It would appear that these types of bacteria, particularly the corynebacteria, do not become established readily under non-body conditions.

Gram Negative Rods

Gram negative rods are an important part of the body microflora, especially in the intestinal tract. Enterobacteriaceae were isolated from most of the body areas cultured and the occurrence of these organisms is recorded in Table 19 . As would be expected, the Enterobacteriaceae were isolated most frequently from the fecal samples and Escherichia coli was the organism that occurred most commonly, being isolated from almost all subjects in almost every sampling period. The outstanding exception was Subject 6 from whom no typical E. coli was isolated. Selected cultures of E. coli were tested for the occurrence of several potentially pathogenic types and in most of the subjects, these types were not found. The notable exception was Subject 5 who showed typable E. coli in six of the nine fecal samples which were cultured from this subject, with Poly A 0111:B4 being specifically found in four sampling periods 4, 5, 6 and 8. An occasional E. coli of a potentially pathogenic type was found in Subject 1 and Subject 3.

Among the other gram negative rods found in the feces of these subjects was an occasional aerobacter and one klebsiella, but the presence of these organisms is common and does not require further comment. The occurrence

of the potential pathogen Salmonella Vi + in the first sample of feces from Subject 3 represents a single isolation of this organism. However, the repeated isolation of shigella Poly B from Subject 6 in samples 4, 6, 7, 8 and 9 appears to indicate that this subject carried this organism fairly regularly during a major part of the test program. This subject also had a typable Bethesda-Ballerup in the first and seventh sampling periods, which finding coupled with the lack of occurrence of E. coli, presents an interesting picture with respect to gram negative rods in the feces of Subject 6. (See Figure 1.)

Shigella was not identified among the cultures isolated from the other subjects and Bethesda-Ballerup was typed only once more from Subject 4 in the second sampling period. Thus it would appear that transference of shigella from one subject to another did not occur in this test, while Bethesda-Ballerup may have been transferred in one instance.

In addition to the recognizable Enterobacteriaceae, two types of gram negative rods were isolated fairly frequently from several subjects during the course of the experimental trial. Both of these organisms did not ferment the sugars in TSI media, and failure to give a positive cytochrome oxidase test indicated that these organisms were probably not pseudomonas. These two strains are referred to in this report as SAD-1 and 2, using the presence (SAD-1) or absence (SAD-2) of a black sediment at the base of the growth on the slant as the primary characteristic of separation of these two types of bacteria from each other. In addition SAD-1 has a peculiar rotten vegetable smell as opposed to SAD-2 which has a sweet smell. The black sediment and unusual odor were constant and pronounced and served as excellent indicators of the presence of these organisms. Using this pattern for differentiation, SAD-1 was isolated from the feces a total of five times from three subjects during the first or second sampling periods. SAD-2 was found a total of five times from four subjects, all during the first or second sampling period with two exceptions. SAD-2 was found once during the second and once during the fifth sampling period on Subject 6, the subject who had previously shown unusual gram negative rods throughout the entire trial.

Enterobacteriaceae were found in other body areas as well as in the feces. The groin area of four subjects frequently showed aerobacter. Subject 1 showed a gram negative rod in eight out of ten sampling periods from the groin, seven cultures of which were aerobacter and one was an E. coli. Subject 3 carried aerobacter in three out of the four initial sampling periods after which it did not occur in further samples from the groin. In addition two subjects each showed a single isolation of SAD-1, Subject 4 in the ninth sampling period and Subject 8 in the tenth sampling period. Enterobacteriaceae were isolated occasionally from the glans penis with E. coli being found in the tenth sampling period on Subject 1 and SAD-1 occurring in sample 4 from Subject 6.

Aerobacter was isolated in five out of the six initial sampling periods from the axilla of Subject 3 while Subject 1 had an aerobacter in the same body area in sample period one only. Subject 4 showed an E. coli in the third sampling period in the axilla and SAD-1 in the ninth, and SAD-1 occurred sporadically in the other body areas during various sampling periods. SAD-2 was isolated from the eye of Subject 8 during the second sampling period.

The cultures selected for identification of the gram negative rods were picked from the MacConkey's agar and the blood agar, both incubated aerobically. In no instance were any of the cultures studied obtained from the anaerobic series.

In summary, the occurrence of the Enterobacteriaceae in the feces of most subjects followed the expected pattern. The notable exceptions were Subject 6 who carried shigella, Bethesda-Ballerup and an unidentified gram negative rod rather than E. coli as the predominating fecal aerobic bacteria, and Subject 5 who persistently carried potentially pathogenic types of E. coli. The occurrence of gram negative rods in other areas of the body was sporadic with the exception that Subject 1 persistently carried aerobacter in the groin and Subject 3 showed the same organism repeatedly in the axilla. The unidentified gram negative rods SAD-1 and 2 occurred occasionally in the feces, throat, buccal area, axilla and glans penis of several subjects.

The experimental conditions did not appear to influence markedly the occurrence of the Enterobacteriaceae in the body areas, although aerobacter occurred less frequently in the axilla and groin of certain subjects in the latter part of the trial. Some minor variations were found with respect to SAD-1 and 2 which were isolated from the feces only in the first two sampling periods, while SAD-1 was found in later sampling periods in the other body areas. These data suggest that the gram negative rods both in the feces and body areas were influenced more by the individual than by the experimental conditions.

The occurrence of gram negative rods in the chamber and cottage isolated from the exposure plates presented contrasting pictures. The gram negative rod patterns SAD-1 and 2 occurred 36 times in the chamber in all four environmental areas tested, beginning with the second sampling period. Only two other gram negative rods were found, including one isolation each of aerobacter and E. coli. However, in the cottage only four total isolations of gram negative rods occurred, three of pattern SAD-1 and one aerobacter. (See Table 20 and 21)

Gram negative rods were also isolated repeatedly from plates made from the wash water. These organisms were predominantly aerobacter and pattern SAD-1. The smallest number of isolations occurred during period two, when the subjects had been recently supplied with clean new wash cloths. It is interesting to note that only one isolation of gram negative rods was made following this from the two subjects in the cottage, whereas the wash cloths from Subjects 1, 2, 3 and 5 in the chamber showed repeated contamination with gram negative rods and Subject 4 and 6 showed such a contamination beginning with the fourth sampling period. Gram negative rods were also isolated frequently from the urine bottles, particularly those in the chamber during the first sampling period when E. coli, aerobacter, and gram negative rod pattern SAD-2 occurred. Following this, only five isolations were made during the remaining two sampling periods. It is interesting that the sample from the urine bottle of Subject 1 who carried aerobacter so consistently on the groin area showed aerobacter only once. (See Tables 22 and 23.)

From these data it is clear that the gram negative rods carried in or on the bodies of the subjects spread to their environment, as the chamber and cottage both showed no gram negative rods until the men had occupied them, after which certain types of gram negative bacteria previously isolated from the men appeared in their environment. The wash cloths and urine bottles were especially heavily contaminated.

Miscellaneous Organisms

The occurrence of PPLO-type organisms from the various body areas is recorded in Table 24 . This type of organism occurred sporadically on all but two of the subjects and was found mainly in the feces with several isolations from the groin and G. P. One subject, 2, showed a single isolation each from the throat and buccal cavity. The isolations occurred mainly during the middle part of the experiment from sampling periods four through eight.

The fungi isolated from the various body areas of each of the subjects for all periods are summarized in Table 25. The most commonly isolated organism on several of the subjects was trichosporum. This organism was found repeatedly throughout the entire sampling period on Subject 3 in the groin area and sometimes in the G. P. This type of fungus was also found occasionally in the other subjects, particularly Subjects 2 and 7, occurring mainly in the groin and G. P. in these subjects as well. Two other types of fungi occurred several times. Aspergillus was found sporadically in the throat, groin and G. P. on three of the subjects and Candida sp. or Candida albicans was found in various body areas on two subjects. Subject 7 carried Candida sp. in the groin and G. P., whereas Subject 6 showed Candida albicans twice in the throat and once in the groin. Penicillium and rhodotorulla were also found, with the later organisms occurring twice in the G. P. of Subject 4. One isolation of cladosporum was made from the throat of Subject 6. The occurrence of the fungi seemed to be more nearly related to the individual subject than to the experimental condition.

Very few fungi were isolated from the exposure plates made from the room areas as shown in Table 26. None was found in the chamber. Candida sp. was isolated twice in the cottage during the fifth and sixth sampling periods and cladosporom was found twice in the cottage in the final sampling period. Candida sp. was carried continuously by Subject 7, a cottage inhabitant; cladosporom was never isolated from either of the subjects living in the cottage. Trichosporum and penicillium each were isolated once from the crotch of the suits prior to donning. The fungi isolated from the body areas and the environment did not appear to interact to any great extent.

No protozoa or spirochetes were seen.

The recovery of chromogenic colonies from the actinomyces plates from the body areas and from the environment was sporadic and the organisms found appeared to be largely Actinomyces albus. Among the subjects, 4 and 7 showed the most numerous isolations, with five and four cultures, respectively, and the personal hygiene areas of both the cottage and chamber had the highest incidence of this type of microorganism with two isolations each. No pattern was noted in their occurrence on the subjects or in their environment. (Tables 27 and 28.)

Types of Bacteria Involved in Buildup

The results of the study of the selected colonies from each of the body areas in each of the sampling periods have shown that a variety of microorganisms were present on the body of the subjects and the increase in total colony counts shown to occur as the experiment progressed indicated that there was a buildup of bacteria. It is important to know which types of bacteria are involved in the buildup. This information was obtained from a microscopic examination of highest dilution showing growth in the aerobic and anaerobic broth series made by serially diluting each sample from the body areas tested, and from an examination of the type of bacterial colonies appearing most frequently on the blood plates. In the broth series, the microorganism found in the cultures representing the highest dilution of the sample is presumed to be the predominating organism and a selective study

of the kind of bacterium associated with the distinctive colony types found in the greatest numbers on the blood plates allows an estimate to be made of the type of predominating bacteria present in the sample inoculated on that plate.

Table 29 presents the results of the microscopic examination of aerobic broth series made from the axilla, groin and G. P.

From these body areas two types of organisms usually predominated - rods resembling corynebacteria and cocci resembling staphylococci or micrococci - morphologically. (For simplicity the term staphylococci will be used throughout this report to represent this morphological type.) A few streptococci were seen also. With few exceptions the organisms seen in the top dilution of the aerobic and anaerobic broth series from the same sample appeared to be the same so the results are presented from the aerobic series only. In most instances the initial samples from the axilla showed a predominance of staphylococci, although two subjects showed rods. In general, staphylococci were the most frequently isolated organism during the middle portion of the trial, but at about the seventh or eighth sampling period three of the subjects in the chamber showed a predominance of rods and Subject 4 showed the predominance of this type of bacterium from the fourth period through the eighth. Half of the subjects retained the rod to the end of the experiment, while the other half showed staphylococci in the last two periods. The men in the cottage showed staphylococci consistently throughout the entire experimental trial with the exception of Subject 7 who showed the rod to be predominant in the last sampling period.

The same two organisms predominated in the groin and five of the subjects showed staphylococci in the initial sample while the other three started with a rod. In the subjects, both chamber and the cottage, the staphylococci and rods alternated in most of the subjects during the middle of the experiment, but about the seventh or eighth period all of the subjects began to show a predominance of the rod over the coccus and this was true for the rest of the experiment.

The predominating organisms observed in cultures from the G. P. were similar to those found in the other two body areas, the groin and the axilla, although streptococci predominated initially in the G. P. of Subjects 3 and 6. Most of the subjects initially showed staphylococci and with the exception of Subject 3 continued to show staphylococci and rods alternately until about the seventh sampling period. Subject 3 carried streptococci or rods continuously throughout the entire experimental period. Beginning with the eighth sampling period and continuing to the end of the experiment, the rod predominated in almost all sampling periods for all subjects.

Thus it would appear that staphylococci or micrococci and rods resembling corynebacteria were the predominating organisms of the axilla, groin and G. P. during the entire experimental trial for all subjects. Staphylococci were slightly more prevalent in the axilla than in the groin or G. P., and were more predominant in the axilla of the two subjects in the cottage than in the six subjects in the chamber, especially during the final sampling periods. The rods resembling corynebacteria were found to be the predominating organisms from about the seventh sampling period onward in the groin and G. P. of the subjects, both in the chamber and the cottage and in the axilla of some of the subjects in the chamber.

During the colony enumeration of the aerobic blood plates, as well as during the observations of the anaerobic blood plates, estimates were made of the predominating organisms by observing the distinctive colony types occurring on the plates. These estimates were based on the correlation of colony appearance with the actual identification of selected bacteria forming these colonies. The aerobic blood plates taken from the axilla, groin, and G. P. rather consistently showed a predominance of colonies that were judged to be staphylococcus; and although some of these plates showed a high concentration of rods which resembled corynebacteria, the rods did not appear to predominate on the aerobic plates as often as on the plates incubated under CO_2 or in the broth series. Interestingly enough when the predominating organisms as judged by colony type were estimated on the CO_2 incubated plates taken from the axilla, groin and G. P., the rods resembling corynebacterium were predominant

in many instances. For example, in samples from the groin the rod was predominant 25 times more often on the CO_2 incubated plates than in the aerobic plates, 13 times more often in the G. P. (even through many samples from the G. P. showed very poor growth) and 12 times from the axilla. Thus, when the CO_2 plates from the last sampling periods were considered the rod predominated in the G. P. in six out of the eight subjects, eight out of eight of the subjects in the groin area and in four out of eight of the subjects in the axilla. The remainder of the subjects showed a predominance of staphylococci in the axilla in the final sampling periods. When comparing the predominant organism found in the broth series with the predominant organism found in the blood plates, there was good agreement on eight out of eight subjects for the axilla and groin areas and six out of eight subjects for the G. P. area. From these data, the buildup of bacteria in the groin and G. P. of all subjects and in the axilla of certain subjects was due to rods resembling corynebacteria. Staphylococci or micrococci were responsible for the buildup in the axilla of the other subjects. Since many of the subjects showed that staphylococci predominated initially, the buildup of corynebacteria in the body areas of several subjects represented a shift in the predominating organism in these men.

Microscopic observations similar to those made from the aerobic and anaerobic broth series of the axilla, groin and G. P. were made from the top dilution cultures of the two broth series from both the throat and buccal cavity to determine the predominating morphological types of bacteria. The results are summarized in Table 30. The streptococci were overwhelmingly the predominating bacteria in both the throat and buccal areas during the entire experimental period. Some staphylococci or micrococci were seen and an occasional rod resembling a corynebacterium was noted. Apparently the buildup of bacteria which occurred in the throat and buccal cavity as the experiment proceeded represents an increase in the numbers of the same types of bacteria that had been predominating from the start of the experiment. This finding was supported by observations of the colonial types appearing on both the aerobic and CO_2 incubated blood plates during the entire experiment.

Room areas. - In addition to the four plates exposed in both the cottage and the chamber, swabs were taken from eight different areas of the chamber and cottage and inoculated into broth. The cultures which grew from each of these areas were gram stained and observed microscopically and the results are recorded in Tables 31 and 32. Since these observations were made from the broth culture representing the greatest dilution of broth, the types of bacteria seen are assumed to be the predominating flora. In the cottage the filter showed the smallest number of positive cultures and in all three periods rods were observed with staphylococcus isolated only once. This was in marked contrast to the other seven areas which showed quite consistent growth during all sampling periods and commonly carried a staphylococcal type culture. This observation was particularly marked during the final sampling periods nine through fourteen. The toilet seat frequently showed gram negative rods and the chair quite consistently showed a slender gram negative rod. Streptococci were found on the transfer lock handle and the water faucet in certain sampling periods.

Staphylococci were the predominating organisms in the chamber from the beginning of the experiment until the end. Streptococci were found quite frequently on the telephone, the two buttons, the chair and the water faucet. The toilet seat showed this organism and gram negative rods. During the thirteenth sampling period gram negative rods and long slender rods were found in several chamber areas.

From these data it is apparent that staphylococci and gram negative rods in certain areas such as the toilet seat, are involved in any buildup of total bacteria noted.

Wash water. - A broth series was made from the wash water five times during the experimental period and microscopic observations were made on the morphological types of bacteria present, as recorded in Table 33. The types of organisms most frequently seen were gram negative rods of the coli type, staphylococci, streptococci and an occasional sarcina during the second sampling period. Staphylococci and gram negative rods were the types of

bacteria involved in the buildup observed as the wash cloth was used for prolonged periods.

Urine bottles. - Similar microscopic studies were made from the broth cultures made from the swabs from the urine bottles. The data in Table 34 show a predominance of gram negative rods and staphylococci and during the first sampling period of streptococci. Since the urine bottles were frequently washed, these observations do not yield data concerning a buildup of bacteria.

Suits. - Three areas of each suit, the crotch, axilla and right foot, were sampled by swab prior to donning. Almost all areas showed bacterial growth in the crotch, where gram negative rods, staphylococci, and streptococci were most frequently isolated. Microscopic observations on the cultures from the axilla area showed staphylococci, streptococci, rods resembling corynebacterium and gram negative rods. Staphylococcus was the main type of organism isolated from the foot area, with streptococcus and gram negative rods also being found frequently. The organism most frequently found on the blood plates held near the suit vents while the subjects were wearing the suits were staphylococcus and streptococcus. (See Table 35.)

Other miscellaneous organisms which were isolated occasionally were neisseria and hemophilus; but these bacteria were found so infrequently that it is clear that these organisms were not involved in any buildup of organisms either from the men or their environment, and were not affected by the experimental conditions.

The summary of all the aerobic and anaerobic studies of the five body areas show that certain bacteria predominate in each body area. Table 36 indicates the four most predominant general types in each body area and Table 34 breaks down this information into specific types of bacteria and includes the less frequently occurring bacteria as well. From these data it is evident that the aerobic bacteria found most frequently on the various body areas of the eight subjects in this study are similar to those described as the normal body flora by most other investigators.

Strict Anaerobes

The frequency of occurrence of strict anaerobes in the body areas and feces tested emphasizes the importance of these bacteria in the fecal flora. One of the important factors to be considered in the study of the fecal microflora is the degree of predominance of anaerobes over aerobes. Accordingly, aerobic plate counts were made from the same series of dilution tubes from which the height of growth under anaerobic conditions was recorded, and these data are summarized in Tables 38 and 39 for each period. This allows an estimation of the numbers of aerobic versus anaerobic bacteria in the same sample of feces. The data from the aerobic plate counts showed that approximately one million to three hundred fifty million aerobes were present in the feces of the subjects in this study, with most samples having a bacterial count of fifty million or less, although several counts, particularly on Subjects 2 and 5 exceeded this number. The occurrence of the higher aerobic counts suggests individual effect rather than influence of experimental conditions. The height of anaerobic growth in every sample was in the ten billionth dilution of feces or above with many showing growth in the dilution representing a trillion per gram of fresh feces. Thus it is clear that the fecal anaerobes are more numerous than the aerobes by a considerable margin, which confirms earlier studies.

A comparison was made between the numbers of aerobic and anaerobic bacteria by recording the number of ten times difference between the aerobic plate counts and the height of growth in the anaerobic dilution series in Table 40 These data are averaged for each subject and are summarized as well as to give an overall average. This overall average shows a 3.4 times difference between the aerobic and anaerobic "counts" which is compatible with what has been found in previous studies. The two subjects, 2 and 5, which showed the highest aerobic counts naturally gave the lowest difference between aerobic and anaerobic bacterial numbers. These data show that the anaerobes exceeded the aerobes in the feces of these subjects by an average of a thousand times.

The anaerobic technique employed in the culturing of the fecal organisms allows the growth of both strict anaerobes as well as facultative anaerobes.

During the course of this study 314 strict anaerobes were isolated and studied compared to 14 facultative anaerobes, so that about 96% of the top dilution organisms studied were strict anaerobes, which is compatible with findings on other similar experimental trials done in the Republic Laboratories.

The published classifications of the fecal anaerobes are inadequate and for this reason a key for distinguishing fecal anaerobes was devised in a NASA study[4] and will be used in this study. This key is defined in the Appendix.

The distribution of the anaerobes isolated from the fecal samples has been recorded in three tables where the data is tabulated individually for each subject in each period (Table 41), as well as showing composite results for each subject in all periods (Table 42), and for all subjects in each period (Table 43) using the NASA key. This allows a consideration of the frequency of occurrence of each of the type cultures of anaerobes both with regard to each subject and to each sampling period. Almost all of the eighteen FA types and the seven GD types were found during the course of the study and with a few notable exceptions the cultures were rather evenly distributed among the subjects. The type cultures FA-1 and FA-15 were found the most frequently followed by FA-3, FA-14, and FA-18. Other organisms isolated in substantial numbers were FA-5, FA-6, FA-12, FA-13 and FA-17. This correlates rather well with the results of the fecal study of normal adult males done under NASA contract NASw-738 [4] as is shown in Table 44 . In general the type cultures occurring the least often and the most frequently were similar in both studies. However, many more GD types, especially GD-3, were isolated during this study than in the NASA work, as were FA-5, FA-13 and FA-18, while the occurrence of FA-8 and FA-12 was slightly lower on this study than on the NASA project.

There was a tendency for two of the subjects to show a flora rather different from that of the other subjects. Subject 4 had a disproportionate number of isolations of FA-1, FA-12, FA-18 and facultative lactobacillus, while showing no GD types, and Subject 7 showed a disproportionate amount of FA-1, FA-14 and FA-15 while also showing no GD types. Subject 8 had numerous isolations of FA-13 and FA-14, while FA-13 was also found frequently

on Subject 3. These data show that there are probably individual variations in the anaerobic fecal flora even when the subjects are eating the same diet and are in close contact as in the chamber and cottage.

When the data were considered according to the distribution of obligate anaerobes in the fecal samples during the various periods, some rather interesting differences became apparent. The most marked was in the occurrence of the GD types of organisms which had been found previously to occur frequently on a space type diet fed to subjects at Wright Patterson Air Force Base under contract AF33(615)-1748[3] but which are found infrequently on the feces of the normal subjects studied under NASA contract NASw-738.[4] Thirty-three isolations of GD types were made during this entire experiment, as shown in Table 43 , only four of which were found during the first four sampling periods with one subject accounting for half of these isolations. However, from the fifth sampling period on to the end of the trial, these GD types of organisms became much more numerous and were isolated a total of 29 times during the last six sampling periods. This trend suggests that diet may have played a role in the occurrence of the GD types.

Several other interesting minor variations were found as follows: FA-2 occurred only during the third through sixth sampling period, and FA-9 and FA-10 were found mainly during the third and fourth sampling periods, FA-12 was found during the fourth through seventh sampling periods, and no FA-5 or FA-6 was found during the first and second sampling periods. Thus the fecal flora was more diverse during the third to the sixth or seventh sampling periods, since during the first two sampling periods less than ten separate types of anaerobes were isolated, while during the fourth and fifth sampling periods fourteen different varieties of obligate anaerobes were found. This dropped off to an average of ten types of different organisms occurring in the seventh, eighth, ninth and tenth sampling periods. A similar increase in diversity of anaerobic microorganisms followed by a decrease in this diversity has been noticed previously under contract AF29(600)-4555[5] during transition periods when the subjects were shifting from one diet to another.

In summary, the fecal flora shows a predominance of anaerobes over aerobes in the order of magnitude of one thousand times, with strict anaerobes representing about 96% of the cultures isolated and studied. The frequency of occurrence of the FA types of obligate anaerobes found in the fecal samples from these eight subjects was somewhat similar to that in the NASA study, except that GD types were isolated during the present study. The data reflected certain individual variations in the occurrence of the fecal anaerobes, particularly with respect to Subjects 4 and 7. The distribution of the fecal anaerobes also seems to be influenced by the experimental conditions, such as diet composition, since the numbers of the GD types of organisms isolated increased after the subjects had been on diet for about two weeks. The anaerobic fecal flora exhibited the greatest diversity of anaerobic types during the mid-period of the experiment, with a simpler flora being present during the first two and last four sampling periods, which may be related to the shifting of types of fecal microflora associated with the transition from one dietary regimen to another. Experimental conditions other than diet did not appear to influence the anaerobic fecal microflora. All of these findings are in accordance with results from previous studies with men and chimpanzees using similar anaerobic culturing techniques in the Republic laboratories.

Although the majority of the strict anaerobes isolated in this study were found in the feces, the occurrence of obligate anaerobes isolated from areas of the body other than the feces have been summarized in Tables 45 and 46. Strict anaerobes were isolated fairly frequently from the glans penis of several subjects, and these bacteria were of an interesting type, which appeared to fit into Bergey's[6] classification for peptococcus. These bacteria occurred in two main patterns, 1 and 2, which are defined by morphology and physiology in footnotes on Table 45. Slight variations in these patterns are also footnoted on the table. Peptococcus 1 was isolated most frequently from the G. P. of Subjects 2 and 5 with all but one culture appearing after the fourth sampling period. Subjects 7 and 8 also showed this culture occasionally. Peptococcus 2 was isolated frequently from the G. P. during the entire experimental period, but occurred most often during sampling periods seven and eight. This culture

was found most frequently on Subjects 3 and 8 although Subjects 1, 2, 4 and 5 showed occasional occurrences of Peptococcus 2 in the G. P. and it was isolated once each from the groin of Subjects 3, 4 and 6. Veillonella was isolated twice from the G. P. of Subject 3 who had shown veillonella several times in the feces, and Eubacterium sp. was isolated sporadically from several subjects, particularly toward the end of the experimental trial.

The occurrence of strict anaerobes in the throat and buccal cavity is summarized in Table 46. Peptococcus 2 was found quite frequently in the throat and buccal cavity of several of the subjects, being isolated three times from Subject 3, who also carried it frequently in the G. P. However, Subjects 5 and 6 showed this organism frequently in the throat and buccal cavity but had shown it only occasionally or not at all from the G. P. Only one isolation was found in the throat and buccal cavity of Subject 8 who carried it prominently on the G. P. The most frequently occurring strict anaerobes in the throat and buccal cavity were veillonella. These organisms were isolated during various sampling periods from all of the subjects and appeared more frequently in the buccal cavity than in the throat. However, numerous isolations were made from the throat samples of several subjects in period five and to a lesser extent in period six. Fusobacteria were isolated sporadically from the throat or buccal area particularly from Subject 5. Other miscellaneous anaerobic organisms isolated were several cultures of strictly anaerobic lactobacillus, bacteroides, and single isolations of Clostridium acidiurici, sphaerophorus and catenabacter. Three FA types of anaerobes were isolated, once each.

From these data it is evident that strict anaerobes inhabit many areas of the body other than the intestinal tract and that the types of organisms isolated often are characteristic of the body area. For example, in this study Peptococcus 1 was found only in the G. P. and veillonella was found largely in the buccal cavity and throat, as were certain types of fusobacter. Peptococcus 2 was more widely spread being found in the G. P., groin (occasionally), throat and buccal areas. The high oxygen atmosphere did not seem to influence the occurrence of the strict anaerobes from the chamber subjects, and with the possible exception of peptococci in the G. P. and veillonella in the throat, the strict anaerobes did not show a buildup.

DISCUSSION OF RESULTS

The microbiological determinations carried out in this study were designed to detect the effect of various conditions typical of space flight including minimum personal hygiene procedures, confinement, space type diets, space suits and 100% oxygen at 5 psia upon the microflora of the subjects and their environment. To do this both aerobic and anaerobic cultural procedures were used to study the kinds and numbers of bacteria present in several body areas, including the axilla, groin, G.P., throat and buccal area, as well as the feces and several areas of the chamber and cottage. The samples were taken frequently throughout the experimental period, at intervals planned to reflect the changing conditions of the experiment, especially the effect of 100% oxygen at altitude to which the six men in the chamber were subjected. Two men living in the cottage served as controls with respect to the 100% oxygen at altitude.

The first question involved the effect of the minimal hygiene procedure coupled with confinement on the numbers of bacteria on the man and in his environment, and both plate counting and serial dilution techniques were employed to determine whether the total numbers of bacteria increased during the trial. Both methods showed that there was a buildup of bacteria in all body areas and in both of the environments as the experiment progressed. The buildup was greatest in the body areas where sweating occurred, the axilla and groin, and was also pronounced in the buccal cavity where the effects of minimal oral hygiene probably were felt. The buildup was greater in the chamber where six men lived than in the cottage, and the numbers of microorganisms in the chamber fluctuated more than in the cottage for an unexplained reason. This buildup usually occurred gradually and then tended to plateau or even to fall off somewhat rather than to continue rising at each testing period. This suggested that more prolonged confinement per se probably would not result in a much greater build up of microorganisms.

The enumeration of the microorganisms was made by the customary aerobic plate counts, except for the feces which will be considered separately. This

procedure led to somewhat erratic results particularly with respect to certain body areas in the latter half of the experiment. A check of duplicate plates made from these body areas, and incubated under CO_2 revealed that the colony counts taken from these plates would have yielded higher counts than the aerobic plates, quite often at the same sampling period when the aerobic counts were lowest, toward the end of the experiment. This suggested that the bacteria present in these body areas at these sampling periods were favored by somewhat anaerobic conditions, and a study of the comparative heighth of growth of the aerobic and anaerobic broth series from the body areas confirmed that the anaerobic series almost always grew more bacteria than the aerobic series. Therefore a more accurate total bacterial count for the body areas may well have been obtained in certain sampling periods by using plates incubated under CO_2. This poses the delicate problem of determining when to use aerobic plate counts vs CO_2 plates, as well as how to compare colony counts taken from the plates cultured by two different methods.

The next important factor to be considered is the types of bacteria involved in the buildup in each body area and the environment. To do this, it is first necessary to establish what bacteria are present initially in these areas and then to follow the increase or decrease of each type of microorganism.

To establish the types of microorganisms present, appropriate microbiological procedures were employed to identify the various bacteria cultured from the body areas and environment. The results of these studies showed that the aerobic microorganisms found on the various body areas were in general in good agreement with those reported in the literature for normal adults and that the microflora of the chamber and cottage reflected to a certain degree the more hardy microorganisms such as staphylococci, or micrococci, streptococci and gram negative rods present on the body of the occupants. Certain types of bacteria such as neisseria and hemophilus often reported as part of the normal flora were isolated infrequently from these subjects, as were certain fungi such as Pityosporum ovale. The failure to culture P. ovale was probably due to the deficiency of an essential oil in the medium. Corynebacterium acnes was not identified among the isolants in this study, probably because the

definition of this organism as an obligate anaerobe was strictly adhered to. Of interest also was the lack of yeasts in the axilla, although these organisms were isolated from other body areas.

The source of cultures selected for identification were the blood agar plates, both aerobic and CO_2 incubated, the agar shakes made from the aerobic and anaerobic broth series and the differential media. Therefore some of the cultures were isolated under aerobic and some under somewhat anaerobic conditions. It was interesting to note that many of the same gram positive cocci were isolated under both conditions, suggesting that on primary isolation even the cultures considered to be aerobic can grow under somewhat anaerobic conditions. Most of the gram positive rods studied except the bacilli were isolated from the CO_2 plates, whereas most of the gram negative rods studied were from aerobic sources.

To determine which of the types of bacteria were involved in the buildup of microorganisms two procedures were used. The characteristics of distinctive colonies found in the greatest numbers on the blood plates were linked to bacteria selected for identification, and using this as a basis, estimates were made of the predominating organism on each plate. To check this estimate, microscopic observations were made to determine the morphological types of bacteria occurring in the highest broth culture in the dilution series of each sample. Excellent agreement was obtained between these two methods and from these observations, it was evident that staphylococci or micrococci and corynebacteria were the organisms involved in the buildup of bacteria in the axilla, groin and G. P., with corynebacteria being the most predominant in the latter part of the experiment in the groin and G. P. and on four subjects in the axillar area. Streptococci with some staphylococci or micrococci were principally involved in the buildup in the buccal area and the throat.

The organisms which built up in the chamber and to a lesser extent in the cottage were staphylococci, gram negative rods and streptococci, probably from the bodies of the occupants.

Because of the aerobic techniques used in enumerating the bacteria, no strict anaerobes were specified as being involved in a bacterial buildup on any of the body areas, excluding the feces and during the studies on strict anaerobes, only the peptococci isolated from the G. P. offer any evidence of increasing in prevalence as the experiment progressed. However, since strict enumeration of the obligate anaerobes is difficult, the increased incidence of this type of bacterium in the G. P. area of several subjects during the last few sampling periods may not indicate a true numerical buildup.

Veillonella were found to be the most frequently occurring strict anaerobe in the throat and buccal cavity and there was an apparent buildup of these bacteria in the throat about the mid-point of the experiment. Fusobacteria were prevalent in the throat and buccal cavity, as was one type of peptococcus. Certain types of strict anaerobes such as lactobacelli, bacteroides and others were isolated occasionally, principally from the throat, buccal cavity and groin. Few obligate anaerobes were found in the axilla and groin.

From the data presented and discussed, it is evident that the minimum hygiene procedures employed in this experiment coupled with confinement did bring about the buildup of microorganisms both in the body areas tested and in the environment, which reached a plateau about midway in the experiment and remained on this plateau or declined. This increase in bacteria seemed to be associated with the microflora usually found in that area of the body and did not seem to harm the subjects. The bacteria in the environment also reached a plateau, after which no further substantial increase occurred and the most numerous bacteria in the environment apparently came from the subjects.

These findings are strengthened by their general agreement with the results of the series of similar tests conducted at Wright-Patterson Air Force Base.[1]

The strict anaerobes isolated in this study were principally from the feces, where they comprise over 95% of the predominating flora. Anaerobes were shown to outnumber aerobes by about 1000 fold and for this reason the work on fecal bacteria in this study emphasizes the strict anaerobes. The techniques for

isolating these organisms and the key for grouping similar bacteria has been devised under NASA contract NASw-738,[4] and the discussion of the results involving the fecal anaerobes will be based on the data from the NASA study on normal males.

In general the fecal anaerobes isolated during this experiment correspond well both with respect to kinds and frequency of distribution to the NASA study. Most of the FA types defined in the NASA experiment were found in this study and the most and least prevalent types were similar on both studies. There was more variation between individuals than between sampling periods in the occurrence of the FA types.

There was one notable point of divergence between the two studies in the increased occurrence on this experiment of strict anaerobes of the GD type, a group of black slime, gas forming proteolytic bacteria that were seldom found in the NASA study. The GD types occurred more frequently in the latter part of the trial in a pattern similar to, but not as marked as that which was found in subjects fed a space-type diet at Wright-Patterson Air Force Base.[3] The pattern in both of these trials suggests that diet was involved in the increase in isolations of the GD types.

Also worthy of comment is the increase in diversity of the fecal anaerobes after the men had been on the space type diet for about a week followed by a decrease in the diversity about two weeks later. This increase in the variety of fecal anaerobes present in a group of subjects has been noted before, during a nutrition study conducted with primates under contract AF29(600)-4555[5]. The increase occurred whenever there was a shift from one diet to another.

Thus the findings with respect to the fecal anaerobes in this study are in good agreement with other similar studies and indicate that diet is the most important factor influencing the fecal anaerobes.

The effect of 100% oxygen at altitude and the wearing of space suits during certain periods in the trial were other experimental variables. There was little, if any evidence that either of these factors influenced either the body, fecal or

environmental microflora. The possible exception was the seemingly smaller fluctuations in the bacterial counts of the axilla and groin of the cottage inhabitants as well as in the environmental bacterial counts in the cottage itself. This may have been due to the smaller number of men in the cottage with the resultant quieter conditions or to a more careful sampling technique practiced by these men.

An important microbiological consideration in the confined environment of a space capsule is the presence of potential or frank pathogens which could affect the well-being of the space travelers. During the course of this study particular attention was paid to the isolation of any such bacteria, and three types of potentially dangerous bacteria were isolated. From the first fecal sample from Subject 6, as well as from the seventh fecal sample, the potentially disease-producing Bethesda-Ballerup was isolated and typed, and this same subject repeatedly showed the pathogen, shigella, during the most of the experiment. The shigella was not found in any other subject, but the Bethesda-Ballerup was isolated from Subject 4 in the second fecal sample. Neither subject appeared to be ill.

The other incident involved the isolation of several coagulase positive, phage typable staphylococci from the bodies of several of the subjects as well as from their environment. One subject developed staphylococcus-infected pustules, but the potentially pathogenic staphylococci were not involved. Thus dangerous bacteria were present throughout the experiment but fortunately in no instance did the possibly dangerous bacteria cause an overt illness in the subject, and these bacteria did not buildup in the subject's bodies or their environment. However, a microbiological examination of the subjects prior to the experiment would have revealed the presence of these bacteria,and proper treatment could probably have been instituted to eliminate the possibility of trouble from these bacteria.

Another important microbiological consideration is the transference of bacteria, particularly pathogens, from one subject to another. In this experiment there were three good opportunities to determine whether bacteria are

transferred, two of which involved the fecal bacteria, Bethesda-Ballerup and Shigella Poly B. There was no transfer of the shigella, but it appears that one instance of the transfer of the Bethesda-Ballerup did occur. However this bacterium apparently did not implant firmly, as it was isolated from the second subject only once.

All coagulase positive staphylococci were studied for phage type and from eight typable cultures, five different phase types were found. No transfer of a phage typable staphylococcus from one subject to another was demonstrated.

Another possible instance of transference involved the peptococci. Peptococcus 1 was found only once (in Subject 5) prior to the fifth sampling period, after which this type of organism was isolated in several subjects. However, this "spread" of Peptococcus 1 from a subject confined in the chamber to two men living in the cottage weakens the transference concept in this instance, unless the transfer was effected in the two weeks prior to the start of the experiment by a light inoculum of Peptococcus 1 that required several weeks to implant firmly.

From these data, it would appear that transference can occur under certain circumstances, but it did not seem to occur frequently nor to have lasting effect in this trial.

Additional studies were made on the bacterial content of the wash water coming from the face cloths of the subjects. During the initial week of the experiment two men shared each of four "space sinks" for washing purposes. When the wash water from these space sinks was tested microbiologically, the numbers of bacteria found coupled with the presence of many coliform organisms contributed to the decision to abandon this method of washing. The substitution of clean face cloths twice during the trial which were rinsed in the regular wash bowl temporarily cleared up this undesirable situation, but as soon as the wash cloths were used continuously, the same unsatisfactory condition developed, although not quite as acutely.

CONCLUSIONS AND RECOMMENDATIONS

Microbiological studies were carried out on several body areas and the feces of eight subjects and their environment at frequent intervals during an experiment to test the effect of minimum hygiene procedures in a confined area, space diets, 100 percent oxygen at 5 psia and the wearing of space suits on these men. Several conclusions can be drawn from this work.

1. The total number of colonies on aerobic blood plates in all body areas and in the environment increased as the experiment progressed. The buildup in the axilla, groin, G. P. and buccal area reached a plateau by the mid-point of the experiment and then stayed relatively constant or decreased, while the buildup in the throat flora was more variable. Although the counts from all body areas fluctuated, consideration of the colony counts from the blood plates incubated under CO_2 from the skin areas would eliminate many of these fluctuations. Bacterial buildup was larger and more irregular in the chamber than in the cottage.

2. The types of microorganisms found in each of the body areas and feces were in good agreement with those regarded in published reports as normal body microflora for that area of the body. Whereas the same types of bacteria usually grew under both the aerobic and anaerobic conditions used in this experiment, several predominating types grew better under CO_2 incubation on primary isolation. The kinds of microorganisms found in the environment reflected the hardier types of body microorganisms isolated from the subjects.

3. The bacteria involved in the buildup of microflora in the axilla, groin and G. P. were staphylococci or micrococci and corynebacteria, with the corynebacteria predominating in the groin and G. P. of the subjects and in the axilla of four of the subjects in the last sampling periods. Streptococci, and to some extent staphylococci or micrococci, were involved in the increase in

microorganisms in the throat and buccal area. The buildup of corynebacteria in most instances represented a relative increase of the rod over the cocci toward the end of the trial while the increase of streptococci seemed only to indicate an increase in the numbers of the type of bacteria that had predominated throughout the experiment. The buildup was most marked in the body areas where sweat is a factor. The strict anaerobes peptococcus and veillonella may have built up in the G. P. and throat, respectively. The bacteria involved in the buildup in the environment were largely staphylococci or micrococci, gram negative rods and, to a lesser extent, streptococci.

4. The buildup of bacteria on the body areas occurred in such a pattern that it appears to be the result of the minimal personal hygiene procedures with the subjects in a confined area and the increase in bacteria was of such a nature that it does not appear harmful for a 34 day period.

5. In the feces strict anaerobes represented over 95 percent of the predominating bacteria and outnumbered the aerobes by more than 1000 to 1. In general, the types of fecal anaerobes isolated, as well as the frequency of their occurrence, agreed well with the distribution of the bacteria described as FA types on the basic NASA study with one significant exception. After the subjects had been on the experimental diet for about two weeks, the type of fecal anaerobes designated as GD started to increase and continued to be isolated frequently for the remainder of the trial. These GD types of organism which form gas, black-slime and are proteolytic, were previously found associated with a space-type diet eaten by subjects on a study conducted by Wright Patterson Air Force Base under contract AF 33(615)-1748[3]. This change in fecal anaerobes probably is diet connected. Another interesting finding was an increase in the diversity of fecal anaerobes, starting about a week after the men changed to the experimental diet and continuing for about two weeks, after which the variety of fecal anaerobes decreased to the original level, a finding similar to that observed in primates when their diet was shifted on a nutrition study conducted by 6571st Aeromedical Research Laboratory at Holloman Air Force Base under contract AF 33(600)-4124[7].

6. Other experimental conditions, such as the 100 percent oxygen at 5 psia and the wearing of space suits did not seem to affect the body flora materially, as there was no marked difference in the microflora of the subjects when these experimental conditions prevailed.

7. The potentially pathogenic bacteria, Shigella Poly B, Bethesda-Ballerup and coagulase-positive phage typable staphylococci were isolated from certain subjects during the experiment; but these bacteria did not cause overt illness and did not appear to transfer readily from one subject to another.

8. Only one apparent transfer of a bacterium from one subject to another occurred when Bethesda-Ballerup was isolated from Subject 4 after having been isolated from Subject 6 previously. This organism did not seem to implant well, as it was isolated only once from the second subject. The Shigella Poly B and phage typable staphylococci did not transfer to other subjects.

9. The types and numbers of bacteria found in the wash water from the "space" sink contributed to the abandonment of the use of these sinks. The microbiological examination of the wash water from the wash cloths used in the latter part of the experiment indicated they were unsatisfactory also when used continuously.

10. The kinds and numbers of bacteria isolated from the neck of the urine bottles indicated that these bottles could serve as a source of undesirable contamination of the environment.

11. The method of cleaning the space suits left a sizeable residual contamination of typical body organisms which could be transferred to the body of the wearer. The air coming from the vent of the space suits during wearing is contaminated, but not excessively, with body microflora.

As the result of a study of the data obtained in this experiment, several recommendations for consideration in future studies will be made.

1. Because the subjects in this type of study may come from widely separated areas, a thorough microbiological study of these men should be done

to detect carriers of potentially pathogenic bacteria before the start of the experiment.

2. A better method of cleaning the face and hands should be devised, as the wash cloth used repeatedly carried undesirable bacteria.

3. A better method of cleaning the space suits is necessary to eliminate cross-contamination of body bacteria between wearers.

4. A better method of handling urine collection is desirable to prevent growth of bacteria in the collection unit that may contaminate the environment.

5. "Total" bacterial counts of the body areas should be made from two sets of blood agar plates, one incubated aerobically and the other under CO_2.

6. Although the buildup of microorganisms on the bodies of the subjects and in the environment did not prove to be excessive or to be harmful under the conditions employed in this 34 day experiment, further microbiological studies of this type will be advisable when trials involving longer confinement or different experimental conditions are undertaken.

APPENDIX A

TECHNIQUES

Collection of Samples

The procedures for the collection of samples from the body areas, feces, environmental and miscellaneous areas are described for each class of samples.

Body areas. - Two swabs from each body area were collected by subjects in the chamber and cottage at 7-8 AM on specified sampling days (see Table 1). One swab was placed in 10 ml of Gall's broth plus cysteine for anaerobic culturing and one was placed in 10 ml of heart infusion broth for aerobic culturing. Collection was made by swabbing the area as follows:

(1) Eye (first sample period only) - Evert lower eyelid and swab confunctiva gently, following contour of eyelid with swab.
(2) Groin - Swab from front toward rear.
(3) Axilla - Swab with care to get specimen from skin below hair area.
(4) Throat - While depressing tongue, swab tonsillar area.
(5) Buccal Area - Swab gingival margin adjacent to the last upper right molar.
(6) Glans Penis - Swab specified area of skin of glans, or between glans and foreskin.

For purposes of approximate quantitation each swab was considered to contain about 0.01 gm of sample.

Feces. - Fecal samples were eliminated into sterile containers and were cultured immediately. Composite samples were taken by inserting a standard loop into five separate areas of the fecal mass, and the 0.01 gm sample was placed into 10 ml Gall's broth plus cysteine, representing a 10^{-3} dilution of the feces. Samples were received as indicated on Table 2.

Environmental areas. - Aerobic cultures were made from several room areas, using two procedures:

(1) Sedimentation plates of blood, MacConkey's, actinomcyces agar, and phytone yeast were made from the following room areas as indicated on Table 1 by exposing the plates for thirty mintues.

- TV
- Table
- Bed
- Personal hygiene area

(2) Swabs were taken from the following areas of the chamber and cottage, placed into 10 ml broth and incubated aerobically as indicated in Table 1.

- Telephone (chamber only)
- Filter (cottage only)
- Toilet seat
- Transfer lock handle
- Two buttons (chamber only)
- Table top (cottage only)
- Water faucet
- Bed post
- Floor area
- Chair

Miscellaneous items. - Cultures were made from the lips of the urine collection bottles, water squeezed from face cloths, from three areas of the suit prior to donning and from the air coming from the suit vents after donning. Samples were taken at the intervals indicated in Table 1.

(1) Urine Bottles - The urine bottles were cultured by swabbing around the outside rim of the urine bottle and placing the swab into 19 ml of Gall's broth plus cysteine, which represented a 10^{-3} dilution of the original samples.

(2) Wash Water - The wash water was cultured by taking 0.5 ml of the squeezed water from the wash cloth and adding it to 10 ml of Gall's broth.

(3) Suit Areas - The suit areas were sampled at the axilla, crotch, and right boot prior to donning by taking two swabs, one of which was placed in 10 ml of Gall's broth plus cysteine for anaerobic culturing and the other in 10 ml of heart infusion broth for aerobic culturing.

(4) Suit Vent - Forty-eight hours following the donning of the suit, samples were taken by holding a blood plate approximately one foot from the vent of each suit for about fifteen seconds. This was done subsequently two more times at approximately weekly intervals.

Primary Culturing

Primary culturing of body areas (other than feces).

Aerobic: The aerobic swab collected by each subject for each body area was emulsified in 10 ml of broth into which it had been placed when collected and serial dilutions in 4-6 tubes were made in heart infusion broth diluting by 1:10, 1:20, or 1:40 depending upon the numbers of organisms expected to be present in the sample based on previous experience. The exact procedure for culturing is shown in Figure 2 . The heart infusion broth series was incubated aerobically and observed for growth at 24 and 48 hours. All cultures showing growth were smeared. Aerobic plates were made on the media listed in Table 47 for each of the body areas by spreading 0.1 ml of broth from the lead tube plus one on the plate using a glass spreader, and an additional blood agar plate was made in the same manner from the lead tube. Aerobic count was taken from a blood plate.

Anaerobic: The anaerobic swab from each body area collected by each subject in the chamber or cottage was emulsified in 10 ml of broth into which the swab was placed when collected and the sample was then serially diluted through 4-6 tubes of Gall's broth containing cysteine by making dilutions of 1:10, 1:20, or 1:40 depending upon the numbers of organisms expected to be found in that particular sample. The procedure which is essentially the same as the

aerobic method is depicted in Figure 2. The cultures were then incubated in a CO_2 anaerobic incubator at 37°C and were observed after 24 and 48 hours for growth. Agar shakes in Gall's agar were made from the top 2 or 3 dilutions showing growth and slides were made on all cultures that showed growth. The agar shakes were then transported from the site of primary culturing to Republic Aviation Corporation's laboratories where the cultures were further studied. Anaerobic Brewer plates were made with 1.0 ml of the appropriate dilution of the throat, buccal and glans penis samples using Gall's agar with cysteine. A blood agar plate, and where indicated a chocolate agar plate, was inoculated with 0.1 ml from the lead tube plus one and spread over the surface of the plate with a sterile, bent-glass rod. A pour plate of Rogosa's agar, when appropriate, was inoculated with 1.0 ml of the lead tube plus one. These plates were incubated in the CO_2 anaerobic incubator. Deep blood agar shakes were made from the buccal sample only by placing 1 ml of blood into a cooled Gall's agar shake and inoculating with 0.2 ml of the lead tube plus one of the buccal sample.

Primary culturing of feces.

Aerobic: The aerobic plates from the fecal sample were taken from the anaerobic broth series. One-tenth ml from the lead tube plus one was spread on one blood plate, and all other aerobic plates listed in Table 47 under media for feces including the second blood plate were made by spreading 0.1 ml of the lead tube plus two on the plate with a glass rod; 0.1 ml of the lead tube plus two was also used as inoculum for a pour plate for the aerobic count. One ml of the lead tube plus two was used as inoculum for the Rogosa's pour plate.

Anaerobic: The anaerobic broth series for the primary culture of the fecal sample was essentially the same as that used previously by Gall, et al.[8] for culturing rumen anaerobes, and which has been recently successfully adapted in the Republic laboratories to the culture of human feces.[9] This is a technique that can be adapted easily for work under field conditions. Figure 3 gives a schematic representation of the primary culturing technique, which is modified to culture from a standard loopful (0.01 gram) of freshly eliminated fecal material. Samples were cultured within fifteen minutes of elimination.

The fecal material on the standard loop was placed directly into a tube containing 10 ml of Gall's broth prepared with two drops of cysteine and one drop of sodium bicarbonate. This tube was considered to represent roughly a 10^{-3} dilution to the fecal contents. Serial dilutions were made into 11 additional tubes containing 9 ml of Gall's broth prepared as above by transferring 1 ml from the inoculated tube into the next tube, etc. The top 10 tubes were labeled 1 to 10 and were incubated anaerobically in a CO_2 incubator until growth occurred usually within 48 hours. Observations were made at 16 and 24 hours and daily thereafter. These ten tubes were considered to approximate a dilution of the sample from 10^{-4} to 10^{-13}. No dilution blanks were used, as each tube containing broth acts as a dilution blank for the next tube in the series. From tubes 5 and 6 pour plates were made into anaerobic Brewer dishes using Gall's medium with cysteine and bicarbonate added.

The top three tubes showing growth were subcultured into agar shakes using Gall's medium to observe the anaerobic or aerobic character of the growth and to preserve the cultures for transport and for purification and study. Each culture was stained by Hucker's modification of the Gram stain and the slide was observed microscopically.

In addition, blood plates were made from the 10^{-3} and 10^{-4} dilution of the fecal sample by the same technique as the aerobic plates from the other body areas and were incubated in the same manner as the anaerobic broth series. Growth was recorded after 24 hours and the plates were treated in the same manner as the aerobic blood plates to be described below.

Primary culturing of environmental areas. - The sedimentation plates made from the several room areas indicated above were exposed for 30 minutes, incubated at 37°C and were observed for growth at the end of 24 hours. The swab cultures taken from the various environmental areas were placed in broth, incubated aerobically at 37°C and smears were made of all broths that grew.

Primary culturing of miscellaneous items.

Urine Bottles: Using the 10^{-3} broth dilution containing the swab, serial dilutions representing 10^{-5} and 10^{-7} dilution were made by taking 0.1 ml

from the previous tube into 10 ml of Gall's broth. In addition 0.1 ml from the 10^{-3} tube was streaked by the usual procedure on MacConkey's plates, Mitis salivarius and two blood plates, one of which was incubated aerobically and one under CO_2. Pour plates using Gall's agar were made using 1 ml of the 10^{-3} and 10^{-7} dilutions as inoculum.

Wash water: Using the lead tube containing the wash water as inoculum, 0.5 ml was transferred serially into three more tubes containing 9 ml of broth. These broths were incubated aerobically. Pour plates were made using Gall's agar inoculated with 1 ml of the first and third tube in the series with Gall's broth.

Suit areas: Using the lead tube as inoculum one more serial dilution was made from each suit area and blood plates were streaked from this dilution from each suit area and incubated aerobically.

Suit vent: The exposure plates made by holding the blood plates 1 foot for 30 seconds were incubated aerobically and were observed for growth.

Subject 4: Approximately one week after donning the suit Subject 4 noticed a bloody discharge near the area of the buttocks and at that time several other pustules were noted on his upper torso near the vent in the suit. Swabs for culture were taken from the pustules in both areas and placed on blood plates.

Secondary Culturing

Aerobic. - All the cultures from the Petri dishes incubated aerobically and under CO_2 from all body areas, feces, environmental areas and miscellaneous items were returned to the Republic Aviation Corporation laboratories and selected colonies were picked into broth. Cultures picked from the anaerobically incubated plates were incubated in the CO_2 incubator while all other colonies from the aerobic plates were processed by the usual aerobic methods. The cultures were smeared, stained, observed microscopically, separated according to morphological types, and processed according to the schema if applicable.

Appendix A

(1) Staphylococci and Micrococci

- Mannitol salt agar
- All positives confirmed with coagulase test
- Phage typing on selected cultures

(2) Streptococci

- Alpha hemolysis
- Beta hemolysis
- Gamma hemolysis
- Differential sugars
- Typing

(3) Pneumococci

- Pneumococcus broth - bile solubility

(4) Haemophilus

- Identified with typing antisera

(5) Neisseria

- Sugar screen test

(6) Lactobacillus

- pH in glucose broth

(7) Gram Positive Rods

- Loeffler's
- Ziehl Neelsen
- Sporulation
- Gelatin
- Sugar screen
- Hydrolysis of starch
- Detection of hyphae (Proact. or Nocardia groups)
- Tellurite
- Catalase
- Hemolysis on sheep blood
- CO_2 requirement

(8) Gram Negative Rods

- TSI
- Indol
- Methyl red
- Voges-Proskauer
- Simmon's citrate
- Urease
- Nitrate
- Litmus milk
- Motility
- Gelatin
- KCN
- Phenylalanine
- Cytochrome oxidase (on all alkaline over alkaline TSI's)
- Typing antisera (Shigella, Salmonella, E. coli, Klebsiella)

(9) PPLO

- Dienes' stained agar technique

(10) Fungi and Actinomyces

- WET mount
- Lactophenol cotton blue
- Corn meal agar
- Fermentation series

(11) Spirochetes

- Blood broth (morphology)
- Darkfield when indicated
- Vincent's stain

(12) Protozoa

- Identification by selective stains

Phage typing: Phage typing of staphylococci isolated in the course of this contract were done by Dr. John E. Blair, Head, Department of Microbiology, Chairman International Subcommittee of Phage Typing of Staphylococcus, The Roosevelt Hospital, Long Island, New York.

The cultures of staphylococci submitted for examination were isolated from various body surfaces of the test subjects or from environmental sources in the chamber and the cottage. The 33 cultures received included 25 coagulase-positive strains and 8 coagulase-negative strains. One culture proved unsuitable for study because of poor growth.

For typing, the 22 standard phages recommended by the International subcommitted on Phage typing of Staphylococcus were used.[10] The phages are: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D, 81 and 187. In addition, four "experimental" phages from Dr. Blair's personal collection were used which sometimes have proved useful for the identification of certain strains that are not typable with the standard phages; these phages were B5, D, 77ad, and UC18.

The methods recommended by the Subcommittee were employed. Cultures were typed first with the routine test dilutions (RTD) of the phages. Those cultures which showed no significant lytic reactions at RTD were then retyped with the phages in concentrations 1000 times stronger than RTD. The phage pattern, or "type", of a culture is reported by listing those phages that produced significant lysis. Cultures showing no significant lysis at either RTD or 1000 x RTD were recorded as nontypable.

Although it is well known that coagulase-negative strains of staphylococci are not susceptible to lysis by the typing phages, the seven viable coagulase-negative cultures nevertheless were submitted to typing together with the coagulase-positive cultures because these strains had been isolated from Subject 4 who had pustules and from Subject 7 because of the high frequency of isolation of staphylococci from this subject. None of the coagulase-negative cultures was typable.

Anaerobic.

Body areas other than feces: The agar shakes made from the dilution series and the colonies picked from the Brewer plate (when made) were separated into two groups depending upon the degree of anaerobiosis. The obligate anaerobes were processed in the same way as the fecal anaerobes described below with the exception that many of the cultures, particularly from the buccal area, throat and glans penis were identified from Bergey's manual[6] rather than from the anaerobic "key". The facultative anaerobes were grouped according to morphology and were processed as indicated for the aerobes of similar morphology.

Feces: The agar shakes from the top three tubes of the cultural series were processed in the following manner. The agar shake cultures were transferred to Gall's broth plus cysteine and incubated anaerobically until growth occurred. Gram stains were made, and if the cultures were pure, they were immediately screen tested as described below. Cultures showing two or more distinct morphological types of bacteria were purified by plating using the following anaerobic technique. A needle of the impure broth culture was spread on a bed of Gall's agar which was then covered with a layer of Gall's agar with added cysteine. The plates were incubated anaerobically in a Torbal jar with hydrogen and 10% CO_2 and discrete colonies were picked. Selected colonies on the anaerobic Brewer dishes originating from tubes 5 and 6 were picked and treated like the subcultures from the agar shakes as described above. The physiological studies of the pure cultures isolated from the feces included the following screen tests:

(1) Gram stain to observe morphology
(2) Final pH in 0.1% glucose broth
(3) Fermentation of the following sugars in Gall's media with glucose omitted (Glucose, Sucrose, Lactose, Dextrin - sugars added at 0.1% level aseptically after autoclaving)
(4) Growth in Gall's broth with no carbohydrate added
(5) Liquefaction of 12% gelatin in Gall's media minus carbohydrate
(6) Growth and reaction in litmus milk (to which 0.05% bovine albumin and 0.1% of peptone have been added)
(7) Growth in agar shake containing Gall's Media

All media contained bicarbonate and all media except the agar shake contained cysteine to produce an Eh of about -200 mv. The results of the screen tests on each anaerobic culture were compared with a "key" derived from tests done on NASA study[4]. This "key" consists of the results of the screen tests from the most frequently occurring fecal anaerobic cultures and is designed to group similar bacteria. Each different screen test pattern is assigned an FA, FN or GD number. The GA and GD types are used to designate obligate anaerobes and the FN types are facultative anaerobes (see Table 48 and Figure 4).

Media Composition

The exact composition of the media used for primary and secondary culturing is included in this section.

Primary media.

BLOOD AGAR PLATE

Purpose: Cultivate fastidious microorganisms

Formula:

Base	Gms/Liter
Infusion from beef heart	10.0
Peptone "M"	10.0
Sodium chloride	5.0
Agar	15.0

pH 6.9

Then add: 5% defibrinated sheep blood

Technique: Streak the plate with the original specimen or a subculture from broth.

Procedure: Incubate 37°C for 18-24 hours

Reaction: Colonies of bacteria usually grow luxuriantly, and the Hemolytic types exhibit clear distinct degrees of hemolysis.

Reference: Difco Manual[11] p. 88

MITIS SALIVARIUS AGAR

Purpose: The detection of fecal streptococci. Incubate exactly 24 hours at 37°C

Formula:

Peptone "M"	20.0 gms/liter
Dextrose	1.0 gms/liter
Sucrose	50.0 gms/liter
Dipotassium Phosphate	4.0 gms/liter
Agar	15.0 gms/liter
Trypan Blue	0.075 gm/liter
Crystal Violet	0.0008 gm/liter

pH 7.0

Technique: Streak the plate with the inoculum.

Reaction:

Streptococcus mitis: small or minute colonies
Streptococcus salivaris: blue (smooth or rough), gum drop colonies 1-5 mm
Enterococcus: dark blue or black raised colonies
Coliform: brown colonies
Pleuro-pneumonia: colorless mucoid colonies

Reference: Albimi Laboratories [12]

PHENYLETHYL ALCOHOL AGAR

Purpose: PEA is a selective medium for the isolation of staphylococci and streptococci from specimens also containing gram negative organisms such as proteus and coli

Formula:

Peptone "CS"	20.0 gms/liter
Sodium chloride	5.0 gms/liter
Phenylethyl alcohol	2.5 gms/liter
Agar	15.0 gms/liter

Add: 5% defibrinated sheep blood

pH 7.3

Technique: Streak the media with a heavy inoculum of the original material or with an inoculating loop of a secondary broth culture.

Reaction: Gram positive organisms will form typical colonies while gram negative organisms found in the same specimen are inhibited.

Reference: Albimi Laboratories[12]

ROGOSA'S SL AGAR

Purpose: SL Agar is a selective medium for the cultivation of oral and fecal lactobacilli

Formula:

	Gms/Liter
Peptone "C"	10.0
Yeast extract	5.0
Monopotassium phosphate	6.0
Ammonium citrate	2.0
*Salt solution	5.0 ml
Dextrose	20.0
Sorbitan Mono-oleate	1.0
Sodium Acetate hydrate	25.0
Agar	15.0
Acetic acid	1.32

pH 5.4

*Salt Solution:

Magnesium sulfate $7H_2O$	11.5 gms
Magnesium sulfate $2H_2O$	2.4 gms
Magnesium sulfate $4H_2O$	2.8 gms
Ferrous sulfate $7H_2O$	0.68 gms
Distilled water	1000.0 ml

Technique: Melt agar then cool in water bath to 45°C. Add a drop of broth culture to agar; then make a pour plate.

Procedure: Incubate under partial anaerobic conditions.

Reaction: Selective for cultivation of lactobacilli

Reference: Difco Supplementary Literature,[13] p. 59

PHYTONE YEAST (BBL)

Purpose: For the isolation of dermatophytes especially T. verrucosum from human and animal specimens.

Formula:

Phytone	10 gms
Yeast Extract	5 gms
Dextrose	40 gms
Streptomycin	.03 gms
*Chloramphenicol	.05 gms
Agar (dried)	17 gms

* Chloromycetin TM Parke Davis & Co.

Technique: Streak slant directly with heavy inoculum of fecal suspension or other suspicious material

Reaction: Typical colonies of the dermatophytes grow rapidly on phytone yeast agar.

Reference: Baltimore Biological Laboratories (14)

MAC CONKEY AGAR

Purpose: Primary differential plating media for coliforms.

Formula:

Peptone "M"	10.0 gms/liter
Lactose	10.0 gms/liter
Bile salts	1.5 gms/liter
NaCl	5.0 gms/liter
Agar	15.0 gms/liter
Neutral Red	0.025 gms/liter

pH 7.1

Technique: With an inoculating loop, streak the plate with the original specimen or subculture from a broth culture.

Procedure: Incubate plate at 35-37° C for 16-18 hours. Prolonged incubation may lead to confusion of results.

Reaction: Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This reaction is due to the action of the acids, produced by fermentation of lactose, upon the bile salts and the subsequent absorption of neutral red. Typhoid, paratyphoid and dysentery bacilli do not ferment lactose and do not greatly alter the appearance of the medium. These colonies are uncolored and transparent.

Reference: Difco Manual,[11] p. 131-132.

DESOXYCHOLATE AGAR

Purpose: Desoxycholate agar is used for the direct enumeration of coliform bacteria. It may also be employed as a non-selective primary plating medium for the isolation of the enteric pathogens.

Formula:

Bacto Peptone	10 gms
Bacto Lactose	10 gms
Sodium desoxycholate	1 gm
NaCl (Sodium chloride)	5 gms
Dipotassium phosphate	2 gms
Ferric citrate	1 gm
Bacto Agar	15 gms
Bacto neutral red	.03 gms

Technique: Streak or smear plate directly with fecal material or with a heavy fecal suspension.

Reaction: Coliform organisms develop red opaque colonies. Colonies of salmonella are colorless and raised with margins varying from smooth to irregular. Shigella colonies are colorless or opaque presenting somewhat of a ground glass appearance.

Reference: Difco Manual[11] p. 63

ACTINOMYCES AGAR

Purpose: To isolate actinomyces

Formula:

Sodium caseinate	2.0 gms
Asparagine	0.1 gms
Sodium propionate	4.0 gms
Dipotassium phosphate	0.5 gms
Magnesium sulfate	0.1 gms
Ferrous sulfate	0.001 gms
Agar	15.0 gms

Technique: Streak the plate with the inoculum

Reaction: Inhibits other organisms and favors actinomyces which can be recognized by typical colony formation and color or smell.

Reference: Difco Supplementary Literature[13]

PPLO

Purpose: Isolation of PPLO

Formula:

Bacto-Beef Heart for Infusions, Infusion from	50 gms
Bacto-Peptone	10 gms
Sodium Chloride	5 gms
Bacto-Agar	14 gms

Technique: Streak the plate with the original specimen

Reaction: PPLO colonies are round with a dense center and a less dense periphery, giving the appearance of a fried egg. They vary from 10 to 500 microns in diameter (0.01-0.5 mm.) and grow into the medium

Reference: Difco Manual[11] p. 89-90

CHOCOLATE AGAR

Purpose: Detection of neisseria, haemophilus, moraxella and other delicate organisms

Formula: Eugon Agar:

Tryptone	15.0
Soy Peptone, Bacteriological	5.0
Sodium Chloride	4.0
Sodium Sulfite	0.2
1-Cystine	0.7
Glucose	5.5
Agar	15.0

To which is added:

5% sterile defibranated sheep blood after agar is cooled to 65°C.

Technique: Streak the plate with the inoculum and incubate under CO_2

Reaction: Colonies have a characteristic form, especially neisseria

Reference: Baltimore Biological Laboratories[14]

DEEP BLOOD AGAR SHAKE

Purpose: To isolate spirochetes and other delicate anaerobes

Formula:

Gall's Agar	9 cc
Sterile defibrinated sheep blood	1 cc

Technique: Inoculate as for agar shake

Reaction: Gram stains from any growth and examine for spirochetes

GALL'S MEDIUM

Purpose: Anaerobic culturing

Formula:

Peptone C (Albimi)	1%
Peptone S (Albimi)	1%
Beef Extract (Difco)	1%
Yeast Extract (Difco)	1%
K_2HPO_4	0.1%
KH_2PO_4	0.1%
Glucose	0.1%

Technique: Make up to 100 ml with distilled water and tube in 9 ml amounts (pipetted for exactness of dilution) and sterilize exactly 10 minutes by autoclaving. Immediately before use, add aseptically 1 drop of sterile 10% $NaHCO_3$ and two drops of 10% cysteine-bicarbonate solution.* This gives a pH of approximately 6.8 and an Eh of approximately -200 mv. Add 1.5% agar to the above when agar is needed for shakes and plates. This is done when originally making the media. In agar omit cysteine except where noted otherwise. To all broth and agar media 0.05% of bovine serum is added.

*10% Cysteine-Bicarbonate Solution

20 gms Cysteine Hydrochloride
100 ml 1N NaOH
7% $NaHCO_3$

Add the cysteine hydrochloride to the NaOH, giving an approximate pH of 7.0.

More or less NaOH will be needed depending on the particular batch of cysteine hydrochloride.

To 4 ml of this solution (15% as cysteine) in a test tube, add 2 ml of 7% $NaHCO_3$. Seal with melted vaspar. Autoclave at 15 lb for 10 minutes.

Secondary Media.

TRIPLE SUGAR IRON (TSI)

Purpose: Preliminary screening of gram negative rods

Formula:

Peptone "M"	20.0 gms/liter
Lactose	10.0 gms/liter
Saccharose	10.0 gms/liter
Dextrose	1.0 gm/liter
Sodium Chloride	5.0 gms/liter
Iron Ammonium Citrate	0.5 gm/liter
Sodium Thiosulfate	0.5 gms/liter
Agar	15.0 gms/liter
Phenol Red	0.025 gms/liter

pH 7.3 ±

Technique: Using needle with inoculum, go into butt first, then zig zag on slant while withdrawing needle from butt. Incubate 20-24 hours.

Reaction: Acid butt (yellow), alkaline slant (red) - Glucose fermented acid throughout medium, butt and slant yellow - lactose or sucrose or both fermented. Blackening of the butt - hydrogen sulfide produced. Alkaline slant and butt (medium entirely red) - none of the three sugars fermented.

Reference: Albimi Laboratories[12]

INDOL BROTH

Purpose: Part of IMVIC schema for identifying Enterobacteriaceae

Formula:

Bacto peptone	20 gms
Sodium chloride	5 gms
Distilled water	1,000 ml

Sterilize at 121° C 15 minutes Add 10 cc/tube

Technique: Inoculate broth and incubate for 48 hours at 37°C

Test Reagent: Kovac's

Pure amyl or isoamyl alcohol	150 ml
Paradimethylaminobenzaldehyde	10 gms
Concentrated pure hydrochloric acid	50 ml

Dissolve aldehyde in alcohol and then slowly add acid. The dry aldehyde should be light in color. Kovac's reagent should be prepared in small quantities and stored in the refrigerator when not in use.

Procedure: Add about 0.5 ml of reagent, shake tube gently. A deep red color develops in the presence of indol.

Reaction: The red color indicates production of indol from the amino acid.

Reference: Edwards & Ewing,[15] p. 248.

Appendix A

METHYL RED-VOGES PROSKAUER BROTH (MRVP)

Purpose: Part of IMVIC schema for identifying Enterobacteriaceae

Formula:

Buffered peptone (Peptone M)	7 gms
Glucose	5 gms
Dipotassium phosphate	5 gms
Distilled water	1,000 ml

Final pH 6.9 - adjust with HCl to 7.1 or 7.2 before autoclaving.

Technique:

MR: Inoculate 5 cc of broth and incubate at 37°C for 5 days.

VP: Inoculate 5 cc of broth and incubate at 37°C for 2 days.

Test Reagent:

MR: Methyl red 0.1 gm
Ethyl alcohol (95 to 96%) 300 ml
*Water - Q.S. to 500 ml

*Dissolve dye in the alcohol and add sufficient distilled water to make 500 ml.

VP: O'Meara (Modified)

Potassium hydroxide	40 gms
Creatine hydrate	0.34 gm
Distilled water	100 ml

Procedure:

MR: Use 5 or 6 drops of reagent per 5 ml of culture. Reactions are read immediately.

Positive tests are bright red.
Weakly positive tests are red-orange.
Negative tests are yellow.

VP: Use reagent in proportion of 1 ml to 1 ml culture. Test may be placed at 37°C or left at room temperature. In either case, final readings after 4 hours. Tests should be aerated by shaking tubes. A positive test turns red.

Reaction:

MR: A positive reaction is indicated by a distinct red color showing the presence of acid. A negative reaction is indicated by a yellow color.

VP: A positive test is indicated by the color showing that the organism produces acetylmethylcarbinol.

Reference: Edwards & Ewing,[15] pgs. 249 and 256.

BACTO-KCN BROTH BASE

Purpose: KCN broth base is recommended for the differentiation of Enterobacteriaceae, particularly to separate the salmonellae from the Bethesda-Ballerup group and to distinguish the klebsiella from Escherichia coli. Moeller showed that media containing potassium cyanide permitted differential growth of Enterobacteriaceae. E. coli, salmonella and shigella were inhibited in the medium while members of the klebsiella, Bethesda-Ballerup and proteus groups grew unrestrictedly. E. freundii also grew in the medium.

Formula:

Proteose Peptone No. 3 Difco	3 gms
Disodium Phosphate	5.64 gms
Monopotassium Phosphate	.225 gm
Sodium Chloride	5 gms
KCN (add 15 cc of .5%)	15 cc

Technique: The tubes are inoculated heavily with 1 to 3 loops of a 24 hour broth culture of the test organisms.

Reaction: Observations for growth are made at the end of 24 and 48 hours incubation.

Reference: Difco Supplementary Literature,[13] p. 122.

MANNITOL SALT AGAR

Purpose: Isolation and identification of Staphylococci

Formula:

Peptone "M"	10.0 gms/liter
Beef Extract	1.0 gms/liter
Sodium Chloride	75.0 gms/liter
d-Mannitol	10.0 gms/liter
Agar	15.0 gms/liter
Phenol Red	0.025 gms/liter

pH 7.4

Technique: Streak the media with heavy inoculum of original material or with an inoculating loop streak from a secondary broth culture.

Reaction: Staphylococci are not inhibited by a concentration of 7.5 per cent sodium chloride. Pathogenic staphylococci produce colonies with yellow zones while nonpathogenic staphylococci produce small colonies surrounded by red or purple zones.

Reference: Albimi Laboratories[12]

LOEFFLER BLOOD SERUM AGAR

Purpose: Loeffler Blood Serum is employed in the cultural diagnosis of diphtheria. The growth of diphtheria bacilli is stimulated and other throat organisms are inhibited by this media.

Formula:

Beef serum	70 gms/liter
Dextrose broth infusion	2.5 gms/liter
Whole egg	7.5 gms/liter

Technique: Inoculate slant with original swab obtained from throat or subculture from broth with aid of inoculating loop. Incubate at 37°C for 18-24 hours.

Reaction: On Loeffler Blood Serum C. diphtheria grows luxuriantly and rapidly, developing morphologically typical organisms, in 12-16 hours.

Reference: Albimi Laboratories[12]

Appendix A

GALL'S GELATIN (i. e. 12%)

Purpose: The use of gelatin in culture media for studies of gelatinolysis (elaboration of gelatinolytic enzymes) by bacteria.

Formula:

Bacto tryptone	10 gms
Bacto peptone	10 gms
Bacto yeast extract	10 gms
Bacto beef extract	10 gms
Monobasic potassium phosphate	1 gm
Dibasic potassium phosphate	1 gm
Serum	1 cc
Gelatin	120 gms

LITMUS MILK

Purpose: Litmus milk is recommended for propagating and carrying stock cultures of the lactic acid bacteria and also for determining the action of bacteria upon milk.

Fomula:

Bacto Skim milk	100 gms
Bacto Litmus	.75 gms

Technique: Inoculate litmus milk from a suspension of the test organism or directly from an isolated colony.

Reaction: Litmus milk may be employed as a differential medium for bacteria on the basis of lactose fermentation, caseolysis, and casein coagulating properties. Litmus has the advantage of being readily reduced by certain bacteria. This reduction of the litmus is useful as a differential aid.

Reference: Difco Manual,[11] p. 192.

* * *

CORN MEAL AGAR

Purpose: Corn meal agar is recommended for the production of chlamydospores by Candida albicans and for the cultivation of phytopathological and other fungi.

Formula:

Corn Meal, Infusion from	50 grms
Bacto Agar	15 gms

Technique: Streak surface of the corn meal plate directly with suspicious material or with a culture that grew on preliminary solution medium.

Reaction: Typical chlamydospores are produced by Candida albicans.

Reference: Difco Manual,[11] p. 246

UREASE BROTH

Purpose: Rough grouping of Enterobacteriaceae into proteus, klebsiella, aerobacter or providence group.

Formula:

Urea	20.0 gms/liter
Monopotassium Phosphate	9.1 gms/liter
Disodium Phosphate	9.5 gms/liter
Yeast Extract	0.1 gm/liter
Phenol Red	0.01 gms/liter

pH 6.8 ±

Technique: A heavy inoculum is emulsified in the broth. Incubate 24 hours. Read at 2, 4, and 24 hour intervals.

Reaction: Urease activity is observed by a change of color in the indicator - from salmon to pink - due to the production of ammonia.

Reference: Albimi Laboratories[12]

* * * *

MOTILITY TEST MEDIUM

Purpose: Part of IMVIC schema for identifying Enterobacteriaceae

Formula:

Beef extract	3 gms
Peptone	10 gms
Sodium Chloride	5 gms
Agar	4 gms

Technique: The medium is inoculated by stabbing through the center of the medium about 1/3 of the length of the media and incubated at 37°C for a total of 48 hours. Read at 8, 24, and 48 hour intervals.

Reaction: Motility is manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as modular outgrowths along the stab.

Reference: Edwards & Ewing,[15] p. 249.

PHENYLALANINE

Purpose: Part of IMVIC schema for identifying Enterobacteriaceae

Formula:

Yeast extract	3 gms
DL-phenylalanine	2 gms
(or L-phenylalanine)	(1 gm)
Disodium phosphate	1 gm
NaCl	5 gms
Agar	12 gms
Distilled water	1,000 ml

Tube and sterilize at 121°C for 10 minutes.

Technique: Inoculate broth and incubate 24 hours at 37°C.

Test Reagent: 10% Ferric chloride

Procedure: 4 or 5 drops of ferric chloride reagent are allowed to run over growth on slant. If phenylpyruvic acid has been formed a green color develops in the syneresis fluid in the slant.

Reaction: The medium is used to test for the deamination of phenylalanine to phenylpyruvic acid.

Reference: Edwards & Ewing[15] p. 252.

SIMMONS CITRATE AGAR SLANT

Purpose: Part of IMVIC schema for differentiation of lactose-fermenting Enterobacteriaceae

Formula:

Sodium Citrate	2.0 gms/liter
Sodium Chloride	5.0 gms/liter
Ammonium Dihydrogen Phosphate	1.0 gms/liter
Dipotassium Phosphate	1.0 gms/liter
Magnesium Sulfate	0.2 gms/liter
Agar	15.0 gms/liter
Brom-Thymol Blue	0.08 gms/liter

pH 6.8±

Technique: Using a loop, inoculate lightly. Incubate at 37°C for 48 hours and read

Reaction: A positive test is indicated by the development of a Prussian blue color in the medium, showing that the organism can utilize citrate as a sole source of carbon

Reference: Albimi Laboratories[12]

OXIDASE TEST

Purpose: This rapid test allows for a convenient differentiation between pseudomonas and other gram-negative, lactose-negative colonies.

Formula:

Reagent

A.	Ethylalcohol 95-96%	100 ml
	Alphanaphthol	1 gm
B.	Distilled water	100 ml
	Para-aminodimethylaniline HCl	1 gm

(Reagent B should be prepared frequently and should be stored in refrigerator when not in use.)

Technique: Nutrient agar slant cultures incubated at 37°C, or at a lower temperature if required are recommended. After incubation two or three drops of each reagent are introduced and the tube tilted so that the reagents are mixed and flow over the growth on the slant.

Reaction: Positive reactions are indicated by the development of a blue color in the growth within two minutes. The majority of positive cultures produce strong reactions within 30 seconds. Any very weak or doubtful reaction that occurs after two minutes should be ignored. Plate cultures may be tested by allowing an equal parts mixture of the reagents to flow over isolated colonies.

Reference: Bailey and Scott,[16] p. 160; Edwards & Ewing, [15] p. 251-2.

NITRATE BROTH

Purpose: Part of IMVIC schema for identifying Enterobacteriaceae

Formula:

Meat Extract	3 gms	
Peptone	5 gms	
Potassium Nitrate	1 gm	
Distilled Water	1,000 ml	Put in 5 cc/tube

Technique: Inoculate broth and incubate 48 hours at 37°C

Test Reagent:

A. Dissolve 8 gms sulfanilic acid in 1,000 ml 5 N acetic acid

B. Dissolve 5 gms alphanaphthylamine in 1,000 ml of 5 N acetic acid

Procedure: Immediately before use equal parts of A and B are mixed and 0.1 ml of mixture is added to each culture. A positive test for reduction of nitrate to nitrite is a red color in few minutes.

Reaction: The red color indicates the reduction of nitrates to nitrites.

Reference: Edwards & Ewing[15] p. 250.

REFERENCES

1. "Biomedical Criteria for Personal Hygiene," AF33(615)-1814, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Dayton, Ohio (in progress).

2. Helvey, W. M., Albright, G. A., Benjamin, G. B., Gall, L. S., Peters, J. M. and Ring, H., "Effects of Prolonged Exposure to Pure Oxygen on Human Performance," NASr-92, RAC393-1, November 1962.

3. Gall, L. S., and Riely, P. E., "Determination of Aerobic and Anaerobic Microflora of Human Feces," AMRL-TR-64-107, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, RAC2174-1, October 1964.

4. "Study of the Normal Fecal Bacterial Flora of Man," NASw-738, Office of Space Sciences, National Aeronautical Space Administration, Washington, D. C. (in progress).

5. "The Influence of Diet on the Normal Fecal Flora of the Chimpanzee," AF29(600)-4555, 6571st Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico, RAC1787-5FR.

6. Breed, R. S., Murray, E. G. D., Smith, N. R., "Bergey's Manual of Determinative Bacteriology," 7th Ed., Williams and Wilkins Co., Baltimore, 1957.

7. "Study of Bacterial Flora of the Alimentary Tract of Chimpanzees," AF29(600)-4124, 6571st Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico, RAC1094-5FR.

8. Huhtanen, C. N., Rogers, M. R., and Gall, L. S., "Improved Techniques for Isolating and Purifying Rumen Organisms," J. Bact., 64, 17-23, 1952.

9. Gall, L. S., and Helvey, W. M., "Culture of Anaerobic Fecal Flora in Men Under Simulated Space Conditions," Bacteriological Proceedings, 1963, Abstracts of the 63rd Annual Meeting, American Society for Microbiology, Cleveland, Ohio, May 5-9, 1963.

10. International Subcommittee on Phage Typing of Staphylococcus, Bull. World Health Organization, 24:771-784, 1961.

11. Difco Laboratories, 'Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures," 9th Ed., Detroit 1, Michigan, 1963.

12. Albimi Laboratories, Inc., 35-22 Linden Place, Flushing, New York, 1964.

13. Difco Laboratories, "Difco Supplementary Literature," Detroit 1, Michigan, 1962.

14. Baltimore Biological Laboratory, 2201 Aisqueth St., Baltimore 18, Maryland.

15. Edwards, P. R., and Ewing, W. H., "Identification of Enterobacteriaceae," 2nd Ed., Burges Publishing Co., Minneapolis, Minnesota, 1962.

16. Bailey, W. R., and Scott, E. G., "Diagnostic Microbiology," The C. V. Mosby Co., St. Louis, Missouri.

TABLES AND FIGURES

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TABLE 1

SCHEDULE OF SAMPLES FROM THE BODY AREAS AND THE ENVIRONMENT

AREAS \ DATE	TEST CONDITION: AMBIENT								ALTITUDE – NO SUITS							ALTITUDE PLUS SUITS													AMBIENT PLUS SUITS							
	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ALL BODY AREAS	1							2			3				4			5				6			7				8			9				10	
ENVIRONMENTAL CHAMBER & CONTROL COTTAGE	1							2		3		4			5	6		7				8		9		10			11		12		13		14		
MISCELLANEOUS SAMPLES WASH WATER OR CLOTHS								1							2							3							4								
URINE BOTTLES										1								2							3												
SUIT	SAMPLES TAKEN FROM SUITS AFTER CLEANING AND PRIOR TO DONNING																																				
SUIT VENT																		1							2							3					

NUMBERS REFER TO SAMPLE NUMBER

CONTROLS WERE AT AMBIENT AT ALL TIMES

TABLE 2

SCHEDULE OF THE FECAL SAMPLES

SUBJECT NUMBER	DATES																																				
	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1		2					3		4					5		6					7					8							9		10	
2	1			2				3			4				5			6				7		8							9	10					
3	1		2					3 *								4					5				6					7		8				9	10 *
4	1		2					3				4			5		6								7		8			9						10 *	
5	1 *				2			3		4						5				6		7		8								9			10		
6	1		2						3			4			5		6						7						8			9				10	
7	1		2					3		4						5		6				7				8						9			10		
8		1		2				3		4							5		6			7		8						9				10			

SUBJECTS ON DEHYDRATED SPACE-TYPE DIET THROUGHOUT ENTIRE EXPERIMENT

NUMBERS REFER TO SAMPLE NUMBER

* NO SAMPLE OBTAINED

TABLE 3

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS FOR ALL SAMPLING PERIODS BY BODY AREA
(Three Zeros Omitted)

Subject #	Axilla Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	116.000	21.100	TNTC	TNTC	1,019,000	700	1,030,000	2,700	2,500	TNTC
2	2,600	TNTC	TNTC	10.100	12,100	TNTC	200	300	1,800	102,800
3	2.000	TNTC	TNTC	20,500	100,500	13,500	202,900	100,500	12,800	52,400
4	7,000	TNTC	TNTC	2,100	5,000	10,000	7,500	5,000	10,100	7,500
5	700	800	1,000	1,500	115,000	3,500	8,000	75,000	50,000	250,000
6	100	TNTC	1,000	25,000	150,000	7,500	300,000	200,000	TNTC	TNTC
7	3,500	TNTC	TNTC	39,500	40,000	TNTC	760,000	TNTC	507,000	10,000
8	400	TNTC	1,000	62,500	230,000	TNTC	70,000	TNTC	TNTC	TNTC

TNTC = Too numerous to count

TABLE 3 (cont'd)

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS FOR ALL SAMPLING PERIODS BY BODY AREA
(Three Zeros Omitted)

Subject #	Groin Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	3,000	12,800	2,500	2,000	600	2,500	1,500	1,700	8,500	85,000
2	400	23,000	TNTC	600	4,500	5,800	1,900	52,500	102,000	11,500
3	200	11,600	5,000	106,200	2,100	50,000	50,100	18,000	24,000	259,000
4	200	TNTC	3,000	11,500	5,000	9,300	5,000	1,500	50,000	70,000
5	300	TNTC	TNTC	51,000	91,000	1,800	30,000	175,000	TNTC	35,000
6	1,000	TNTC	12,000	1,002,000	TNTC	3,500	TNTC	80,000	32,000	13,000
7	4,000	TNTC	TNTC	TNTC	TNTC	2,500	5,000	150,000	TNTC	123,000
8	0	TNTC	TNTC	TNTC	TNTC	200	35,000	TNTC	TNTC	50,000

TNTC = Too numerous to count

TABLE 3 (cont'd)

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS FOR ALL SAMPLING PERIODS BY BODY AREA
(Three Zeros Omitted)

Subject #	Buccal Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	21,000	2,500	2,000	10,800	3,500	1,200,000	1,030,000	209,000	151,000	TNTC
2	2,500	11,000	TNTC	35,500	13,000	60,000	TNTC	36,000	253,000	156,000
3	1,600	10,000	4,000	10,000	25,000	116,000	25,000	TNTC	127,000	TNTC
4	22,000	50,000	15,000	130,000	5,000	750,000	7,000	400,000	TNTC	TNTC
5	10,700	11,000	TNTC	15,500	50,000	5,000	50,000	10,000	50,000	TNTC
6	200	1,800	2,600	127,500	252,000	14,000	77,000	3,500	754,000	1,000
7	1,000	2,200	7,600	30,000	135,000	27,000	100,000	40,000	50,000	78,000
8	900	3,300	7,800	8,500	111,000	5,000	50,000	10,000	1,000	100,000

TNTC = Too numerous to count

TABLE 3 (cont'd)

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS FOR ALL SAMPLING PERIODS BY BODY AREA

(Three Zeros Omitted)

Subject #	Throat Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	161,000	26,000	2,800	9,500	40,000	570,000	6,000	41,000	1,000	600,000
2	20,400	16,000	TNTC	110,000	27,000	70,000	540,000	46,000	300,000	66,000
3	20,000	34,500	4,500	6,500	153,000	38,000	15,000	154,000	444,000	6,000
4	12,000	56,000	5,500	22,500	293,000	31,000	3,000	81,000	155,000	1,000
5	10,100	1,700	4,700	50,000	19,000	14,000	4,000	30,000	28,000	32,000
6	8,800	900	500	2,000	103,000	425,000	301,000	5,000	0	3,000
7	24,000	13,000	28,500	1,004,000	31,000	1,000	60,000	76,000	60,000	18,000
8	300	20,000	50,000	35,000	18,000	25,500	105,000	150,000	105,000	90,000

TNTC = Too numerous to count

TABLE 3 (concluded)

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS FOR ALL SAMPLING PERIODS BY BODY AREA
(Three Zeros Omitted)

Subject #	G. P. Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	100	1,300	20,000	107,000	500	101,000	501,000	17,000	447,000	TNTC
2	100	16,000	TNTC	25,000	90,000	300,000	80,000	24,000	103,000	505,000
3	100	TNTC	5,000	TNTC	TNTC	25,000	90,000	1,000	1,000	TNTC
4	3,200	TNTC	50,000	8,000	11,000	38,000	9,000	15,000	22,000	12,000
5	4,000	400	0	50,000	TNTC	35,000	200,000	TNTC	TNTC	60,000
6	0	2,000	20,000	50,000	45,000	2,000	TNTC	70,000	120,000	6,000
7	7,000	100	2,000	32,500	36,000	13,000	30,000	30,000	1,000	502,000
8	200	TNTC	TNTC	1,000,000	20,000	10,000	30,000	4,000	TNTC	30,000

TNTC = Too numerous to count

TABLE 4

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS, ALL SAMPLING PERIODS
(Three zeros omitted)

Subject	Body Area	Sampling Period									
		1	2	3	4	5	6	7	8	9	10
1	Axilla	116,000	21,100	TNTC	TNTC	1,019,000	700	1,030,000	2,700	2,500	TNTC
	Groin	3,000	12,800	2,500	2,000	600	2,500	1,500	1,700	8,500	85,000
	Buccal	21,000	2,500	2,000	10,800	3,500	1,200,000	1,030,000	209,000	151,000	TNTC
	Throat	161,000	26,000	2,800	9,500	40,000	570,000	6,000	41,000	1,000	600,000
	G.P.	100	1,300	20,000	107,000	500	101,000	501,000	17,000	447,000	TNTC
2	Axilla	2,600	TNTC	TNTC	10,100	12,100	TNTC	200	300	1,800	102,800
	Groin	400	23,000	TNTC	600	4,500	5,800	1,900	52,500	102,000	11,500
	Buccal	2,500	11,000	TNTC	35,500	13,000	60,000	TNTC	36,000	253,000	156,000
	Throat	20,400	16,000	TNTC	110,000	27,000	70,000	540,000	46,000	300,000	66,000
	G.P.	100	16,000	TNTC	25,000	90,000	300,000	80,000	24,000	103,000	505,000
3	Axilla	2,000	TNTC	TNTC	20,500	100,500	13,500	202,900	100,500	12,800	52,400
	Groin	200	11,600	5,000	106,200	2,100	50,000	50,100	18,000	24,000	259,000
	Buccal	1,600	10,000	4,000	10,000	25,000	116,000	25,000	TNTC	127,000	TNTC
	Throat	20,000	34,500	4,500	6,500	153,000	38,000	15,000	154,000	444,000	6,000
	G.P.	100	TNTC	5,000	TNTC	TNTC	25,000	90,000	1,000	1,000	TNTC
4	Axilla	7,000	TNTC	TNTC	2,100	5,000	10,000	7,500	5,000	10,100	7,500
	Groin	200	TNTC	3,000	11,500	5,000	9,300	5,000	1,500	50,000	70,000
	Buccal	22,000	50,000	15,000	130,000	5,000	750,000	7,000	400,000	TNTC	TNTC
	Throat	12,000	56,000	5,500	22,500	293,000	31,000	3,000	81,000	155,000	1,000
	G.P.	3,200	TNTC	50,000	8,000	11,000	38,000	9,000	15,000	22,000	12,000
5	Axilla	700	800	1,000	1,500	115,000	3,500	8,000	75,000	50,000	250,000
	Groin	300	TNTC	TNTC	51,000	91,000	1,800	30,000	175,000	TNTC	35,000
	Buccal	10,700	11,000	TNTC	15,500	50,000	5,000	50,000	10,000	50,000	TNTC
	Throat	10,100	1,700	4,700	50,000	19,000	14,000	4,000	30,000	28,000	32,000
	G.P.	4,000	400	0	50,000	TNTC	35,000	200,000	TNTC	TNTC	60,000
6	Axilla	100	TNTC	1,000	25,000	150,000	7,500	300,000	200,000	TNTC	TNTC
	Groin	1,000	TNTC	12,000	1002,000	TNTC	3,500	TNTC	80,000	32,000	13,000
	Buccal	200	1,800	2,600	127,500	252,000	14,000	77,000	3,500	754,000	1,000
	Throat	8,800	900	500	2,000	103,000	425,000	301,000	5,000	0	3,000
	G.P.	0	2,000	20,000	50,000	45,000	2,000	TNTC	70,000	120,000	6,000

TABLE 4 (concluded)

TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR ALL SUBJECTS, ALL SAMPLING PERIODS
(Three zeros omitted)

Subject	Body Area	Sampling Period									
		1	2	3	4	5	6	7	8	9	10
7	Axilla	3,500	TNTC	TNTC	39,500	40,000	TNTC	760,000	TNTC	507,000	10,000
	Groin	4,000	TNTC	TNTC	TNTC	TNTC	2,500	5,000	150,000	TNTC	123,000
	Buccal	1,000	2,200	7,600	30,000	135,000	27,000	109,000	40,000	50,000	78,000
	Throat	2,000	13,000	28,500	1004,000	31,000	1,000	60,000	76,000	60,000	18,000
	G.P.	7,000	100	2,000	32,500	36,000	13,000	30,000	30,000	1,000	502,000
[illegible]	Axilla	400	TNTC	1,000	62,500	230,000	TNTC	70,000	TNTC	TNTC	TNTC
	Groin	0	TNTC	TNTC	TNTC	TNTC	200	35,000	TNTC	TNTC	50,000
	Buccal	900	3,300	7,800	8,500	111,000	5,000	50,000	10,000	1,000	100,000
	Throat	300	20,000	50,000	35,000	18,000	25,500	105,000	150,000	105,000	90,000
	G.P.	200	TNTC	TNTC	1000,000	20,000	10,000	30,000	4,000	TNTC	30,000

TNTC = Too numerous to count

TABLE 5

COMPARATIVE HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES FROM FIVE BODY AREAS

Period 1

Body Area		Subject Number								Average
		1	2	3	4	5	6	7	8	
Buccal	A	2	2	3	3	4	3	4	5	3.3
	AN	5	6	6	6	6	5	4	3	5.1
Throat	A	2	4	4	3	5	2	3	3	3.3
	AN	6	6	6	6	6	6	5	6	5.9
Axilla	A	2	2	3	3	2	0	0	0	1.5
	AN	4	3	3	4	2	L	0	L	2.1
Groin	A	L	2	L	1	5	3	3	1	2.0
	AN	5	4	5	4	3	4	5	1	3.9
G.P.	A	L	0	0	L	0	0	0	0	0.1
	AN	L	1	1	4	L	L	2	2	1.4
Period 2										
Buccal	A	5	5	6	5	3	4	4	4	4.5
	AN	6	6	6	6	6	5	6	5	5.8
Throat	A	4	6	6	7	5	5	6	6	5.6
	AN	3	7	5	6	5	7	6	6	5.6
Axilla	A	1	3	4	4	4	4	4	4	3.5
	AN	4	4	4	4	3	4	1	4	3.5
Groin	A	3	4	3	5	4	5	4	3	3.9
	AN	5	4	4	5	5	5	5	5	4.8
G.P.	A	3	2	4	3	1	3	3	2	2.6
	AN	4	3	4	4	4	4	4	4	3.9

Number = Number of tube in dilution series
L = Lead tube
A = Aerobic
AN = Anaerobic

TABLE 5 (cont'd)

HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES

Period 3

Body Area		Subject Number								
		1	2	3	4	5	6	7	8	Average
Buccal	A	4	5	6	7	5	6	5	4	5.3
	AN	5	6	6	6	6	5	6	6	5.8
Throat	A	3	5	5	5	5	4	6	5	4.8
	AN	6	6	7	3	7	6	3	7	5.6
Axilla	A	2	2	4	4	3	4	3	4	3.3
	AN	4	3	4	4	4	4	2	3	3.5
Groin	A	4	L	3	5	5	3	5	5	3.9
	AN	5	5	3	5	5	5	3	5	4.5
G.P.	A	4	1	2	3	-	2	3	1	2.0
	AN	4	4	4	3	4	3	4	4	3.7
Period 4										
Buccal	A	4	4	4	4	3	4	4	5	4.0
	AN	5	5	6	4	4	3	4	4	4.4
Throat	A	4	4	4	2	4	4	4	4	3.7
	AN	4	5	4	5	4	2	4	5	4.1
Axilla	A	3	3	4	2	4	3	3	4	3.3
	AN	3	3	4	3	2	4	4	4	3.4
Groin	A	5	4	5	5	5	5	4	4	4.6
	AN	3	3	2	4	4	4	3	3	3.3
G.P.	A	2	2	3	4	2	2	4	3	2.8
	AN	2	2	4	3	4	4	4	4	3.4

TABLE 5 (cont'd)

HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES

Period 5

Body Area		Subject Number								Average
		1	2	3	4	5	6	7	8	
Buccal	A	5	3	4	3	4	3	4	3	3.6
	AN	4	4	4	5	4	3	4	3	3.9
Throat	A	2	4	3	3	4	3	4	5	3.5
	AN	2	4	4	3	5	3	4	5	3.7
Axilla	A	2	1	2	2	4	3	4	3	2.6
	AN	2	4	3	4	4	3	3	4	3.4
Groin	A	3	4	4	4	4	2	1	2	3.0
	AN	4	3	3	4	4	4	4	3	3.6
G.P.	A	3	3	4	2	2	2	3	2	2.6
	AN	2	1	3	3	3	2	3	3	2.5
		Period 6								
Buccal	A	3	5	3	3	3	3	4	4	3.5
	AN	4	4	3	4	4	3	4	4	3.7
Throat	A	1	4	3	3	4	3	3	4	3.1
	AN	3	3	3	3	4	3	4	4	3.4
Axilla	A	2	2	2	2	3	3	1	3	2.3
	AN	1	3	4	2	4	6	2	3	3.1
Groin	A	1	4	4	4	4	4	3	4	3.5
	AN	4	3	3	3	3	4	5	3	3.5
G.P.	A	3	3	3	2	4	2	3	3	2.9
	AN	4	3	3	2	4	2	2	4	3.0

TABLE 5 (cont'd)

HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES

Period 7

Body Area		Subject Number								
		1	2	3	4	5	6	7	8	Average
Buccal	A	3	5	3	3	4	3	4	4	3.6
	AN	4	4	3	4	4	3	4	4	3.8
Throat	A	1	4	5	3	4	3	3	4	3.4
	AN	3	3	3	3	4	3	4	4	3.4
Axilla	A	2	2	2	2	3	3	1	3	2.3
	AN	1	3	4	2	4	4	2	3	2.9
Groin	A	4	4	3	4	4	4	3	3	3.6
	AN	3	3	3	3	5	4	3	3	3.4
G.P.	A	3	3	3	2	4	2	3	3	2.9
	AN	4	3	3	2	4	2	2	4	3.0
Period 8										
Buccal	A	3	4	3	3	4	3	3	3	3.3
	AN	3	5	4	3	4	5	4	3	3.9
Throat	A	3	4	3	3	3	3	4	4	3.4
	AN	2	4	3	3	4	3	4	4	3.4
Axilla	A	2	3	4	3	4	4	2	3	3.1
	AN	4	3	3	2	4	3	3	3	3.1
Groin	A	4	4	4	4	4	4	4	4	4.0
	AN	2	5	3	3	5	4	4	3	3.6
G.P.	A	4	3	2	3	4	2	3	3	3.0
	AN	3	3	3	2	4	2	3	4	3.0

TABLE 5 (concluded)

HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES

Body Area		Subject Number 1	2	3	4	5	6	7	8	Average
Period 9										
Buccal	A	4	3	3	3	4	3	4	3	3.4
	AN	4	3	3	4	4	2	3	4	3.4
Throat	A	4	4	4	3	3	3	3	4	3.5
	AN	4	4	3	3	4	4	4	4	3.7
Axilla	A	3	4	3	3	3	3	3	3	3.1
	AN	3	3	3	3	4	3	2	2	3.9
Groin	A	3	4	4	4	4	5	4	4	4.0
	AN	3	4	3	4	4	4	3	5	3.7
G.P.	A	3	3	3	3	4	2	3	2	3.9
	AN	3	2	3	2	4	3	3	4	3.0
Period 10										
Buccal	A	4	4	4	4	3	3	4	3	3.6
	AN	4	4	4	3	3	4	4	4	3.7
Throat	A	1	4	3	3	3	2	4	5	3.1
	AN	2	4	3	3	5	4	4	5	3.7
Axilla	A	4	4	4	4	4	4	4	2	3.7
	AN	4	3	3	2	4	4	3	3	3.3
Groin	A	4	4	3	4	4	4	4	3	3.7
	AN	5	3	4	5	5	4	5	3	4.3
G.P.	A	3	3	3	2	3	2	3	4	2.9
	AN	3	3	4	4	4	3	2	4	3.4

L = Lead tube
A = Aerobic
AN = Anaerobic
Number = Number of tube in dilution series

TABLE 6

SUMMARY OF THE AVERAGE HEIGHT OF GROWTH IN AEROBIC VERSUS ANAEROBIC BROTH DILUTION SERIES FROM FIVE BODY AREAS

Body Area		Average Tube Growth Height
Buccal	A	3.8
	AN	4.4
Throat	A	3.7
	AN	4.2
Axilla	A	2.7
	AN	3.2
Groin	A	3.6
	AN	3.9
G.P.	A	2.6
	AN	3.0

A = Aerobic

AN = Anaerobic

TABLE 7

DILUTION REPRESENTED BY EACH TUBE OF BROTH SERIES FOR ALL BODY AREAS EXCEPT FECES
(three zeros omitted)

Sample Period	Dilution Tube					
	1	2	3	4	5	6
1, 2 & 3	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
4	1/20	1/400	1/8,000	1/160,000	1/3,200,000	1/64,000,000
5 thru 10	1/20	1/800	1/32,000	1/1,280,000	1/51,200,000	1/2,048,000,000

See Figure 3.

TABLE 8

AVERAGE TOTAL BACTERIAL COUNTS FROM FIVE BODY AREAS FOR THE CHAMBER AND COTTAGE SUBJECTS FOR ALL SAMPLING PERIODS
(Three Zeros Omitted)

Axilla

Subjects	1	2	3	4	5	6	7	8	9	10
Chamber	21,500	TNTC	TNTC	29,033	234,350	8,283	303,400	63,917	21,200	110,450
Cottage	1,950	TNTC	<1,000	51,000	135,000	TNTC	415,000	TNTC	278,750	130,000

Groin

Chamber	850	TNTC	TNTC	193,633	32,367	28,817	18,067	54,708	53,083	79,750
Cottage	2,000	TNTC	TNTC	TNTC	TNTC	1,350	20,000	162,000	TNTC	86,500

G. P.

Chamber	1,250	8,620	24,167	56,667	54,250	83,500	180,000	32,833	190,500	263,000
Cottage	3,600	8,000	25,000	516,250	2,800	11,500	30,000	17,000	225,000	266,000

Buccal

Chamber	8,333	14,383	8,933	54,888	58,083	324,167	359,833	176,477	339,167	TNTC
Cottage	950	2,750	7,700	19,250	123,000	16,000	75,000	25,000	25,500	89,000

Throat

Chamber	38,466	22,517	3,533	33,417	105,833	191,333	144,917	59,500	154,667	118,000
Cottage	12,150	16,500	39,250	519,500	24,500	13,250	82,500	113,000	87,500	54,000

TNTC, when used in average, was considered to be equivalent to the highest count obtained in that period.

TABLE 9

TOTAL BACTERIAL COUNT FROM FOUR ENVIRONMENTAL AREAS IN THE CHAMBER*

	Sampling Period													
Area	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Personal Hygiene	2	32	28	22	77	TNTC	33	129	19	12	35	104	28	13
Table	4	25	50	11	91	TNTC	38	120	8	32	100	109	52	29
Bed	-	15	20	5	25	56	2	140	35	9	28	105	51	39
T.V.	-	58	55	25	100	TNTC	44	170	23	27	100	106	38	67

TOTAL BACTERIAL COUNT FROM FOUR ENVIRONMENTAL AREAS IN THE COTTAGE*

Personal Hygiene	54	150	51	101	-	18	30	100	60	69	25	26	48	44
Table	54	54	50	50	-	15	-	-	28	28	-	35	26	30
Bed	-	31	20	25	-	15	-	-	29	20	-	25	30	26
T.V.	-	51	-	52	-	15	-	-	29	50	28	21	30	25

TNTC = Too numerous to count
*Plates exposed 30 minutes

TABLE 10

TOTAL BACTERIAL COUNT FROM SAMPLES OF WASH WATER

(Per ml of wash water)

	Sample Number									
	*1		2		3		4		5	
Subject	Colony Desig.	Colony Numbers	Colony Desig.	Colony Numbers	Colony Desig.	Colony Numbers	Colony Desig.	Colony Numbers	Colony Desig.	Colony Numbers
1	(12)	TNTC	(1)	600	(12)	TNTC	(1)	320	(12)(3) (4)	TNTC
2			(12)	800	(12)	TNTC	(12)	TNTC	(12)	TNTC
3	(12)	TNTC	(12)	200	(12)	TNTC	(12) (1)	80 400	(12) (1)	TNTC 300
4			(1)	2200	(12) (5)	TNTC 2,000	(12) (1)	1500 400	(3) (12)	TNTC TNTC
5	(12)	TNTC	(1)	200	(1) (12)	600 80	(1) (12)	600 20	(12)	TNTC
6			(12)	200	(12) (1)	1,400 60	(12) (1) (5)	60 900 40	(12)	TNTC
7	(12)	TNTC	(1)	400			(1)	20	(1)	600
8			(1)	600	(4) (1)	1,000 600	(1) (4) (1)	600 200 240	(1) (4)	50,000 800

* Space sinks shared by Subjects 1&4, 2&3, 5&6, 7&8 during first sampling period only.
TNTC = Too numerous to count; Numbers in parenthesis refer to probable type of bacterium.
(1) & (4) - Staphylococcus or micrococcus; (5) Streptococcus; (12) Gram negative rod; (3) Corynebacteria

TABLE 11

TOTAL BACTERIAL COUNT FROM URINE BOTTLES

(per swab)

Subject Number	Colony Designation	Number of Colonies
1	(12)	TNTC
2	(12)	1,000,000
3	(12) (1)	320,000 3,000,000
4	(12)	TNTC
5	(12)	TNTC
6	(12)	TNTC
7	(1) (3)	250,000 150,000
8	(13) (6) (2)	300,000 150,000 10,000

(1), (2) & (4) - Staphylococcus or micrococcus; (3) & (6) - Corynebacteria; (12) &(13) - Gram negative rod
Numbers in parenthesis refer to probable type of bacterium
TNTC = Too numerous to count

TABLE 12

TOTAL BACTERIAL COUNT FROM THREE AREAS OF SUIT PRIOR TO DONNING

(Per swab)

Suit Area	Subject Number							
	1	2	3	4	5	6	7	8
Axilla		200,000 (1)				500,000 (1)	700,000 (1)	
Crotch	800,000 (1)	10,000,000 (1) 1,000,000 (6)			1,500,000 (2) 100,000 (4)	250,000 (1)		100,000 (1)
Right Foot	600,000 (1) 100,000 (4)				2,500,000 (1) 200,000 (4)		1,500,000 (1)	400,000 (1)

Numbers in parenthesis refer to probable type of bacterium
(1), (2), (4) - Staphylococcus or micrococcus; (3) & (6) - Corynebacteria;

TABLE 13

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 1

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			G.P., E, Bu. (Ax., Bu.)	(Ax.)			Th.	(Th.)		
2	Gr.	Ax.	G.P.(Gr.)	(Bu.)	Ax., Bu.		Bu., (Bu, Th.)		Th.	
3		Ax, Gr.	G.P., F, (Gr, Th, G.P)	Th.		(Bu.)	Bu.		Th, Bu.	
4		Ax, Gr.	G.P., Bu. (Gr.)	Gr. G.P.	Th.		(Bu.)		Th, Bu.	
5		Ax, Gr.	Gr., G.P.	(Ax, Gr, Th)					Th, Bu.	
6	Ax.	Ax, Gr., G.P.	Ax, Bu.	(Gr.) Gr., G.P.	Bu.		Th.			
7		Gr.	Ax.	G.P., Th.			Th, Bu (Th.)			
8		Ax.	Ax., G.P., F.	Gr. (Ax.)		Bu, Th.	Bu. (Th.)		Th, Bu.	
9	Gr.		Ax, Gr. G. P.	(Ax.)			Th, Bu (Th.)			
10		Ax, G.P.	Ax, Gr.	(Gr.)	G.P.	Th	Bu. (Bu.)			

MSA = Acid on mannitol salt agar; Coag = Coagulase; MS = Mannitol salt

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 2

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			Ax, Gr., E (Gr., Ax.)	Th (Th.)		(Th.)	(Bu.)	(Bu.)	Gr, Th G. P, Bu	
2		Gr.	Ax., Gr., G. P.	G. P. (Bu.)		Th. (Th.)	Bu.		Th., Bu.	
3			Gr., G. P.	Th., Bu. (Gr, Ax.)		Th. (Bu.)	Th. (Th, Bu)		Bu.	
4		Gr., G. P.	Ax., G. P.	Gr. (Th.)	Th.		Th.		Bu.	
5		Gr., Th., Bu.	Ax., G. P. (Ax.)		Bu.		(Bu.)		Th., Bu.	
6	G. P.	Gr., Bu.	Bu.	Bu. (Ax.)			Th, Bu. (Bu.)			
7	Ax.*	G. P.	Gr.	Gr., Th. Bu(Gr., Bu., Ax.)		Bu.	Th, Bu.			
8		G. P.	Gr.	Th. (Ax.)		(Th.)	Th, Bu.			
9		Ax.	Ax., Gr.	Th. (G. P.)			Th, Bu., (Th, Bu)			
10	Ax., G. P.	Gr., G. P.	Ax., Th.	Gr., Th.		Th. (G. P.)	Bu. (Th, Bu)		Bu.	

* Positive phage type

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 3

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			G.P. (G.P.)	Bu. (Bu.)		(Bu., Th.)		(Bu.)	Th. Bu.	
2		G.P.	Ax., Gr., G.P.			Th. (Th., G.P.)	GP, Bu, Th.	Bu. (Bu.)	Th. Bu.	
3			Ax., Gr.	Th. (Bu., Gr.)		Th. (Th., G.P.)	Bu. (Bu.)		Th.	
4		Gr.	Ax., Gr, Bu. G.P.	Gr., G.P. (Th., G.P.)	Th.	(Bu, Th, GP)	(Bu.)	F	Th. Bu.	
5			Ax., Gr. (Ax.)	G.P.					Th.	
6			Ax, Gr, G.P. (Gr.)	Gr., G.P. (Ax.)			Th., Bu (Bu.)			
7			Ax., Gr. (Ax., G.P.)	G.P., Th., (G.P.)	Ax., Gr.		Bu. (Bu, Th)	Bu.		
8			Ax., Gr. (Ax, Bu, Gr)	G.P., Th.			Bu. (Th.)	Th.	Th.	
9			Ax., Gr, GP (G.P.)			Th. (Th.)	Bu. (Bu, Gr)	Bu.		
10		Ax.	Ax., Gr. Th.	G.P. (Gr.)		(Bu.)	Bu.		Th.	

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 4

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			Gr., G.P., F	Ax. (Th.)		Bu. (Th., Bu.)	(Gr.)		Th.	
2		Gr.	Ax.	G.P. (G.P., Th, Bu)		Bu., Th.	Bu. Th.		Th. Bu.	
3			Ax., G.P. (Ax.)	Th. (Th., Gr.)		Th. (Th., Bu.)	Bu. (Bu.)			
4		Gr.	Ax.	G.P. (G.P.)	Bu.	Th.	(Th.)		Th. Bu.	
5	Gr.		Ax., G.P.		Bu.				Th. Bu.	
6		Bu.	Ax., Gr., Bu. (Bu.)	Gr., G.P.		Bu. (Th.)	Th., Bu.	Bu.	Th. Bu.	
7		Gr.	Ax., G.P., Bu.	F, Th.		Bu.	Th. (Th., Bu)			
8			Ax, Gr.	Th.	Bu.	(Bu.)	Th., Bu. (Th.)	F		
9			Ax., Gr. (Gr.)	Th.		Bu. (Bu.)	(Th.)			
10			Ax., Gr., (G.P.)	Ax., Gr.		Th. (Th., Bu.)	Th., Bu.		Th.	

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 5

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1				GP, Bu, Th, Ax. (Gr, Th, GP)		(Bu., Th.)				
2			Ax.	Ax, Bu, Gr, GP (GP, Th, Gr)	G.P.	(Th.)	(Bu.)		Gr.	
3			Bu.	Ax, Th, Bu, Gr. (Ax, Bu, GP)		(Th.)	Th. (Bu.)			
4			Gr, GP, Th.	Th., Bu. (Ax., Th.)		(Bu.)	Th., Bu.	Bu.		
5			(Ax)GP, Ax. Gr.	Gr.	G.P.	Th. (Th.)				
6			Th, GP, Gr, Ax(Ax, Th)				Bu.		Th.	
7			Ax. (Ax.)	Gr, Ax, GP (Gr, G.P.)			(Th.)			
8			Th., Gr., Ax.	Gr, GP, Th (Gr, Th, G.P.)			(Th.)		Bu.	
9			Ax.	GP, Th, Bu, Gr (Th, Bu, G.P.)			Th., Bu.			
10			Ax., Bu.	G.P., Gr. Th.			Th., Bu.		Bu.	

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 6

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			Bu.	Th., Ax. (Bu., Th.)		(Bu., Th.)				
2			G.P., Ax, Gr.	Bu., Gr. (Th, Gr, Bu.)			Bu(Bu, Gr, Th)			
3	Gr.		Ax., G.P.	Th., Bu. (Bu., G. P.)		(Bu.)	Th. (Th.)			
4			GP, Th, Gr.	Th., Bu. (Bu., Gr.)		Th. (Bu., Th.)	Bu.	Bu.		
5			GP, Ax, Gr. Th.	(Gr.)		Th.			Bu.	
6		Gr.	Ax., G.P. (Ax.)	(Gr.)			Bu. (Bu.)			
7		Gr.	GP, Th, Ax.	Bu., Th., Ax. GP (Gr.)			(Th.)		Th. Bu.	
8			GP, Gr, Ax. (Ax.)	Th., Bu. G.P.		G.P.	Bu.			
9		Gr.	G.P., Ax.	Th., Bu. (Th., Bu.)						
10		Gr.	G.P.	Ax., Th.		(Th.)	Bu. (G.P.)	Bu.		

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (cont'd)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 7

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			Bu.	Gr. (Ax., Th.)			Gr., GP		Bu.	
2		G.P.		Bu., Gr. (Th., Gr.)	(G.P.)	(Bu.)	(Th.)		Bu. G.P.	
3	Ax., Gr.			Bu. (Th., G.P.)	(G.P.)	(Bu., G.P.)		Th.	Bu.	
4			G.P.	Gr, GP, Bu. Th.		Gr.	(Bu, Th)		Bu.	
5	Gr.		Ax., Th., GP (Ax.)	Bu. Th.		Th.	(Th.)		Bu.	
6	Ax.*, G.P.	Gr.	Th. (Ax.)	Gr.			Bu. (Bu.)			
7		Ax., GP, Gr.	Th. (Ax., Th.)	(G.P.)			(Bu.)			
8			Ax.	Gr., Bu., Th (Gr.)			Bu. (Bu, Th)			
9		Ax.	Ax.	GP, Th, Bu, Gr. (Gr., G.P.)			Bu. (Bu.)			
10		G.P.	Gr., Ax.	Th.				Th.		

*Positive phage type

Note: Brackets indicate cultures are from anaerobic series

TABLE 13 (concluded)

OCCURRENCE OF GRAM POSITIVE COCCI ON BODY AREAS

Subject 8

Sample Period	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			G. P.	Bu, Th, Ax. (Th, Gr, Bu.)	(G. P.)	Th. (Th.)	Th.	(Bu.)	Th.	
2		Gr.	Ax.	E, Th., G. P., (Gr, Th, Bu.)	(G. P.)		(Bu.)			
3		Ax., Bu.		Th, Gr, G. P. (Ax.)	(G. P.)	(Bu., Th.)	(Bu.)			
4		Gr.	Ax. (Ax.)	Th, Ax, Bu. (Bu, Gr, G. P.)		Th., Bu. (G. P.)				
5			GP, Ax., Bu, Th, Gr, (Ax.)	Gr. (Bu.)		Th.	Th. (Th.)			
6		Th.	Th, Gr, Ax. (Ax.)	Th, G. P. (Th.)	(G. P.)					
7		G. P.	Ax. (Ax.)	Bu., Gr.					Th. Bu.	
8		Gr.	Ax. (Ax.)	Bu, GP, Th. (Gr.)		(Th.)	Bu. (Bu.)			
9			G. P., Ax.	Th, Bu, Gr.		(Bu., Th.)	Th., Bu. (Th.)			
10			G. P.	Ax., Bu.		(Bu., Th.)		Th.	Bu.	

Note: Brackets indicate cultures are from anaerobic series

TABLE 14

PHAGE PATTERN OF COAGULASE POSITIVE STAPHYLOCOCCI

Sample Period	Date	Source of Isolation	Phage Pattern
6	4/1	Subject 7 - Axilla	B5
9	4/7	Cottage - Table	7/47/53/54
9	4/7	Cottage - TV	B5
7	4/8	Subject 2 - Axilla	D
11	4/12	Cottage - TV	7/47/53/54
12	4/14	Chamber - TV	6/47/54/75
12	4/14	Chamber - Bed	7/53/77
14	4/18	Cottage - Table	B5

TABLE 15

OCCURRENCE OF GRAM POSITIVE COCCI IN FOUR CHAMBER AREAS

(Exposure Plates)

Room Area	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
Personal Hygiene	14,	1, 9, 10, 13, 11	2, 3, 4, 6, 13	1, 13	1					
Table	14,	2, 9, 10, 11	2, 3, 4, 6, 9, 11, 14	5, 8, 4, 9						
Bed	6, 14, 13 12*	6, 8	2, 3, 7, 10, 11							
T. V.	13, 12*	2,	2, 3, 4, 6, 7, 8, 9, 10, 11, 13,	5, 9						

MSA = Acid on mannitol salt agar; Coag = Coagulase; MS = Mannitol salt

* Positive phage type

Numerals represent the number of the sampling period.

TABLE 16

OCCURRENCE OF GRAM POSITIVE COCCI IN FOUR COTTAGE AREAS

(Exposure Plates)

Room Areas	Staphylococcus			Micrococcus	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid		salivarius	mitis	enterococcus	α	β
Personal Hygience			*1, 4, 5, 6, 7, 8, 14	1, 3, 4		13, 14	13		
Table	9*, 14*	6,	1, 2, 3, 4, 5, 9, 10, 11, 12, 13	1, 10, 11			5, 14	14	
Bed	14	2, 7, 8, 9	2, 3, 4, 6, 7, 8, 9, 10	5, 11			14		
T. V.	1, 6, 12 9*, 11*	2, 4, 9	1, 4, 5, 8 9, 10, 13, 14	7, 12					

MSA = Acid on mannitol salt agar
Coag.= Coagulase
MS = Mannitol salt
Numerals represent the number of the sampling period.
* Positive phage type

TABLE 16 (concluded)

OCCURRENCE OF GRAM POSITIVE COCCI IN WASH WATER

Subjects	Staphylococcus			Micrococcus	Sarcina	Streptococcus				
	MSA + Coag. +	MSA + Coag. -	MS Growth No Acid			salivarius	mitis	enterococcus	α	β
1			2, 4, 1		5					
2			2							
3			1, 4, 5							
4		4,	2, 4,							
5			5, 2, 4,	5						
6				2, 4, 5						
7			5	5, 2, 4						
8			5	2, 4, 5						

Numbers indicate sampling period

MSA = Acid on mannitol salt agar; Coag. = Coagulase; MS = Mannitol salt

TABLE 17

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 1

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	Bu, Th, F	F					F, Th, Bu			
2	Th, F									
3	F							Gr.		
4										
5	F		Gr.	G. P.				F		
6	Th							G. P., Th, Gr.		
7						Gr		G. P.		
8	F									
9	F	F	G. P.			G. P., Gr				Ax.
10	Th						F, Gr	F		

* Variety of C. striatum that ferments glucose
** pseudodiptheriticum

Ax = Axilla
Bu = Buccal
F = Feces
G. P. = Glans Penis
Gr = Groin
Th = Throat

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 2

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	F									
2	Th, Bu.	F						Gr, F	Ax.	
3				Gr.				Ax.		
4			G. P.					Ax, Gr, G. P.		
5								Ax, G. P.		
6								Gr, G. P.		
7		Gr.						G. P., Ax Bu, Gr.		
8		Gr.						Gr, Ax.		
9			Gr, G. P.	Gr.				Ax.		
10										

* Variety of C. striatum that ferments glucose
** pseudodiptheriticum

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 3

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	F									
2			Gr				G. P.	Ax, G. P.	Gr	
3						Bu		G. P., Gr		
4						Ax		G. P.		
5								Gr.		
6								G. P.		
7	Bu									
8	F							Gr., F		
9						G. P.		G. P.		Ax.
10							Gr.	G. P.	Gr, G. P.	

* Variety of C. striatum that ferments glucose

** pseudodiptheriticum

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 4

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	Th, F									
2	F							Gr, G. P.		
3										
4	F	F								
5										
6	F									
7										
8							F	Gr.		
9										Ax.
10								G. P.		

* Variety of C. striatum that ferments glucose

** pseudodiptheriticum

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 5

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1										
2									Bu.	
3	F	Gr.								
4	Th, F		Gr.							
5							Gr.			
6									Gr.	
7	F									
8										
9	F									
10										

* Variety of C. striatum that ferments glucose
** pseudodiptheriticum

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 6

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	F									
2	F									
3	F									
4							Gr.			
5	F									
6							Gr.			
7									Ax.	
8										Ax.
9	F							Ax.		
10										

* Variety of C. striatum that ferments glucose

** pseudodiptheriticum

TABLE 17 (cont'd)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 7

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	F	F								Ax.
2	F	F		G. P.						
3	F	G. P.								
4		Gr.								Ax.
5									Ax.	
6									Ax.	
7		Ax.								
8			Gr.							
9	F									Ax.
10										

* Variety of C. striatum that ferments glucose

** pseudodiptheriticum

TABLE 17 (concluded)

OCCURRENCE OF GRAM POSITIVE RODS BY SUBJECT FOR EACH SAMPLING PERIOD

Subject 8

Sample Period	Lacto-bacillus	Bacil-laceae	Corynebacterium						Pattern	
			striatum	S+*	Psd**	xerosis	enzy-micum	Sp.	A	X
1	Th.									
2		F, Gr.								
3	F						F			
4						G. P.				
5									Gr, Ax.	
6								Th.		Ax.
7	F									
8			Ax.							
9						G. P.				Ax.
10		Th.								

* Variety of C. striatum that ferments glucose

** pseudodiptheriticum

TABLE 18

PATTERNS FOR CORYNEBACTERIA DESIGNATED AS 'A' AND 'X'

Unidentified Pattern No.	Gelatin Liquifaction	Growth on Gelatin	Nitrate	Fermentation			
				Glucose	Sucrose	Maltose	Starch
A	-	+	-	-	-	-	-
X	-	-	-	-	-	*	-

* Not used in tests for X

TABLE 19

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 1

	Sampling Period				
Body Area	1	2	3	4	5
Feces	E. coli-no type	E. coli-no type E. coli-Poly B* SAD-2	E. coli-no type	E. coli-no type	
Groin	Aerobacter	Aerobacter	Aerobacter		Aerobacter
G. P.					
Misc.	Axilla - Aerobacter A				

	6	7	8	9	10
Feces	Aerobacter	E. coli-no type	E. coli-no type	E. coli-saline +	E. coli-no type
Groin	Aerobacter		Aerobacter	Aerobacter	E. coli-no type
G. P.					E. coli-no type
Misc.					Throat-SAD-1

* Poly B 0128:B12

** SAD-1 TSI alk/alk with black sediment (rotten vegetable smell)

*** SAD-2 TSI alk/alk (sweet smell)

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 2

Body Area	Sampling Period 1	2	3	4	5
Feces	E. coli-no type SAD-1	E. coli-no type SAD-1	E. coli-no type	E. coli-no type	E. coli-no type
Groin					
G. P.					
Misc.					

	6	7	8	9	10
Feces	E. coli-no type	E. coli-no type E. coli-saline +	E. coli-no type	E. coli-no type	E. coli-no type
Groin					
G. P.					
Misc.		Buccal- SAD-1			

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 3

Body Area	Sampling Period 1	2	3	4	5
Feces	Salmonella-Vi +	E. coli-Poly *A & B	No sample received	E. coli-no type	
Groin	Aerobacter A		Aerobacter A	Aerobacter	
G. P.					
Misc.	Axilla-Aerobacter	Axilla-Aerobacter	Axilla-Aerobacter	Axilla-Aerobacter	

	6	7	8	9	10
Feces	E. coli-no type	E. coli-no type	E. coli-no type	E. coli-Poly B no further type	No sample received
Groin					
G. P.					
Misc.	Axilla - Aerobacter				

* Poly A 0111:B4
Poly B 0126:B16, 0128:B12

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 4

Body Area	Sampling Period 1	2	3	4	5
Feces	E. coli-no type	E. coli-saline + E. coli-no type Beth.-Ball.* SAD-1	E. coli-no type	E. coli-no type	
Groin					
G. P.					
Misc.			Axilla- E. coli-no type		

	6	7	8	9	10
Feces	E. coli-no type	E. coli-no type	E. coli-no type	Klebsiella	No sample received
Groin				SAD-1	
G. P.					
Misc.	G. P. - SAD-1			Axilla-SAD-1	

*Bethesda-Ballerup

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 5

	Sampling Period				
Body Area	**1**	**2**	**3**	**4**	**5**
Feces	No sample received	E. coli-Poly A E. coli-no type	E. coli-no type	E. coli-no type E. coli-Poly A 0111:B4	E. coli-Poly A E. coli-Poly A 0111:B4
Groin					
G. P.					
Misc.					

	6	**7**	**8**	**9**	**10**
Feces		E. coli-Poly A 0111:B4	E. coli-Poly A & B 0111:B4		E. coli-Poly B no further type
Groin					
G. P.					
Misc.					

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 6

	Sampling Period				
Body Area	**1**	**2**	**3**	**4**	**5**
Feces	Bethesda-Ballerup	SAD-2		Shigella-Poly B	SAD-2
Groin					
G. P.				SAD-1	
Misc.					

	6	**7**	**8**	**9**	**10**
Feces	Shigella-Poly B	Shigella-Poly B Bethesda-Ballerup	Shigella-Poly B Saline + Shigella	Shigella-Poly B	
Groin					
G. P.					
Misc.					

TABLE 19 (cont'd)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 7

Body Area	Sampling Period 1	2	3	4	5
Feces	E. coli-no type SAD-1	E. coli-no type SAD-2	E. coli-no type E. coli-saline +	E. coli-no type	E. coli-no type
Groin					
G. P.					
Misc.	Throat- SAD-1 Aerobacter				

	6	7	8	9	10
Feces	E. coli-no type	E. coli-no type	E. coli-no type		
Groin					
G. P.					
Misc.		Buccal- SAD-1			

TABLE 19 (concluded)

OCCURRENCE OF GRAM NEGATIVE RODS ON BODY AREAS

Subject 8

Body Area	Sampling Period 1	2	3	4	5
Feces	E. coli-no type SAD-1	SAD-2	E. coli-no type	E. coli-no type	E. coli-no type
Groin					
G. P.					
Misc.	Buccal- SAD-1	Eye- SAD-2			

Body Area	6	7	8	9	10
Feces		E. coli-no type	E. coli-no type		
Groin					SAD-1
G. P.					
Misc.					

TABLE 20

OCCURRENCE OF GRAM NEGATIVE RODS IN FOUR CHAMBER AREAS
(Exposure Plates)

Area	Sampling Period						
	1	2	3	4	5	6	7
Table		SAD-2 SAD-1	SAD-2	SAD-1		SAD-2	SAD-1
TV		SAD-1 Aerobacter SAD-1					SAD-1
Personal Hygiene		SAD-2	SAD-1	SAD-1	SAD-2	SAD-1	SAD-1
Bed							

	8	9	10	11	12	13	14
Table		SAD-1		SAD-1	SAD-1	E. Coli	SAD-1
TV	SAD-2				SAD-1 SAD-2	SAD-1	SAD-1
Personal Hygiene	SAD-1	SAD-1			SAD-1	SAD-1	SAD-1
Bed	SAD-1	SAD-1		SAD-1	SAD-1	SAD-1	

SAD-1 TSI alk/alk with black sediment (rotten vegetable smell)
SAD-2 TSI alk/alk (sweet smell)

TABLE 21

OCCURRENCE OF GRAM NEGATIVE RODS IN FOUR COTTAGE AREAS
(Exposure Plates)

	Sampling Period						
Area	1	2	3	4	5	6	7
Table		SAD-1			Aerobacter		
TV							SAD-1
Personal Hygiene							
Bed							

	8	9	10	11	12	13	14
Table							
TV							
Personal Hygiene						SAD-1	
Bed							

SAD-1 TSI alk/alk with black sediment (rotten vegetable smell)

TABLE 22

OCCURRENCE OF GRAM NEGATIVE RODS IN WASH WATER

Subject No.	Sample Number				
	1*	2	3	4	5
1	Aerobacter SAD-1		SAD-1 Aerobacter		SAD-1
2	Aerobacter SAD-1	Aerobacter	Aerobacter SAD-1	Aerobacter SAD-1	Aerobacter
3	Aerobacter		SAD-1	SAD-1	Aerobacter
4				Aerobacter SAD-1	Aerobacter SAD-1
5	SAD-1	SAD-1	SAD-1		SAD-1
6				Aerobacter SAD-1	SAD-1
7	Aerobacter				
8				Aerobacter	

SAD-1 TSI alk/alk with black sediment (rotten vegetable smell)

* Space sinks shared by Subjects 1 and 4, 2 and 3, 5 and 6, 7 and 8 during first sampling period only

TABLE 23

OCCURRENCE OF GRAM NEGATIVE RODS IN URINE BOTTLES

Subject No.	Sample Number				
	1	2	3	4	5
1	E. coli-Saline + Aerobacter				
2	Aerobacter				
3	SAD-2		E. coli-no type		
4	E. coli-no type SAD-2	E. coli-no type SAD-1			
5	E. coli-no type SAD-2				
6	Aerobacter				
7			SAD-1		
8		E. coli-Saline +			

SAD-1 TSI alk/alk with black sediment (rotten vegetable smell)
SAD-2 TSI alk/alk (sweet smell)

TABLE 24

DISTRIBUTION OF PPLO IN BODY AREAS

Subject Number	Sampling Period	Body Area
1	5 6	Feces Feces
2	5 7	Buccal, Feces Throat
3	5 4 10	Feces Groin Groin
4	None	
5	6	G. P.
6	7	Feces
7	None	
8	6 7 7 8	G. P. Feces Groin* Feces**

* No distinct periphery
** Many atypical

TABLE 25

FUNGI ISOLATED ON PHYTONE-YEAST EXTRACT AGAR

Sample Period	Subject 1	Subject 2	Subject 3	Subject 4
1				
2			Groin - Trichosporum G. P. - Trichosporum	Groin - Candida sp.
3		Groin - Trichosporum	Groin - Trichosporum G. P. - Trichosporum	
4		Axilla - Trichosporum Groin - Trichosporum	Groin - Trichosporum	
5	G. P. - Trichosporum		G. P. - Trichosporum Groin - Trichosporum	Throat - Aspergillus
6	Feces - Penicillium		Groin - Trichosporum	G. P. - Rhodotorula
7			Groin - Trichosporum G. P. - Trichosporum	G. P. - Rhodotorula
8			Groin - Trichosporum	
9		G. P. - Trichosporum	Groin - Trichosporum	Groin - Aspergillis
10			Groin - Trichosporum	

TABLE 25 (concluded)

FUNGI ISOLATED ON PHYTONE-YEAST EXTRACT AGAR

Sample Period	Subject 5	Subject 6	Subject 7	Subject 8
1		Throat - Cladosporium	Throat - Aspergillis	G. P. - Aspergillis
2			G. P. - Candida sp.	
3			G. P. - Trichosporum	
4			G. P. - Candida sp. Groin - Cnadida sp.	
5			G. P. - Trichosporum Groin - Candida sp.	Buccal - Penicillium
6		Buccal - C. albicans		
7				
8		Groin - C. Albicans	Groin - Candida sp.	
9		Buccal - C. albicans		
10			Groin - Trichosporum	

TABLE 26

FUNGI ISOLATED ON PHYTONE-YEAST EXTRACT AGAR
FROM ROOM AREAS AND SUITS

Sample Period	(Exposure Plates) Room Area	Swabs from Suit Prior to Donning
1		
2		
3		
4		
5	Cottage-Personal Hygiene Candida Sp.	Subject 2-Crotch-Trichosporum Subject 6-Crotch-Penicillium
6	Cottage-Personal Hygiene Candida Sp.	
7		
8		
9		
10		
11		
12		
13	Cottage-Bed-Cladosporum Cottage-Table-Cladosporum	

TABLE 27

CHROMOGENIC COLONY RECOVERY FROM ACTINOMYCES PLATES FROM BODY AREA
ACTINOMYCES ALBUS

Subject	Sampling Period											
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2		Groin										
3					Axilla							
4	Eye Axilla			Feces	Throat				Feces			
5							Axilla					
6										Groin		
7			Feces	Axilla			G. P. Groin					
8						Groin						

TABLE 28

CHROMOGENIC COLONY RECOVERY FROM ACTINOMYCES PLATES FOR ROOM AREAS
ACTINOMYCES ALBUS

Area	Sampling Period													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cottage	Personal Hygiene Area					Bed Table						Personal Hygiene Area		
Chamber				Personal Hygiene Area		Table Personal Hygiene Area								

TABLE 29

TYPES OF ORGANISMS FOUND IN HIGHEST DILUTION OF AEROBIC SAMPLE SHOWING GROWTH IN AXILLA, GROIN AND G. P.
(Microscopic Observation)

Subject 1

Body Area	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
Axilla	Rod	RC	Staph	Staph	Staph	Staph	Staph	RC	Strep RC	Staph
Groin	RC	Staph	Staph	RC	Staph	RC	RC	RC	RC	RC
G. P.	Staph	Staph	Staph	Staph	Staph	Staph	Staph	RC	RC	Staph

Subject 2

Axilla	Staph	Staph	Staph	Staph RC	Staph RC	Staph	RC	RC	RC	RC
Groin	Staph	Staph	Staph	RC	RC	Staph	Staph	RC	RC	RC
G. P.		Staph	Staph	RC	Staph RC	RC	Staph	RC	RC	RC

Subject 3

Axilla	RC	RC	Staph	RC	Staph	Staph	Staph	Staph	Staph	RC
Groin	RC	RC	RC	RC	RC	Staph	RC	RC	RC	RC
G. P.		Strep	Strep	RC	RC	RC	RC	Strep	RC	RC

Subject 4

Axilla	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph
Groin	Staph	Staph	Staph	Staph	Staph	Staph	Staph	RC	RC	RC
G. P.	Staph	Staph	Staph	Staph	Staph	Staph	Staph	RC	RC	RC

Rc= Rod corynebacterium; Staph = Staphylococcus; Strep = Streptococcus; F. R. = Fat rods

TABLE 29 (concluded)

TYPES OF ORGANISMS FOUND IN HIGHEST DILUTION OF AEROBIC SAMPLE SHOWING GROWTH IN AXILLA, GROIN AND G. P.
(Microscopic Observation)

Subject 5

Body Area	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
Axilla	Staph	Staph	Staph	RC	RC	RC	RC Staph	RC	Staph	Staph
Groin	Staph	Staph	Staph	RC	RC	Staph	RC	RC	RC Staph	RC
G. P.		Staph	RC	RC	Staph	RC	RC	RC	RC	Staph

Subject 6

Axilla	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	FR	FR
Groin	Staph	Staph	Staph	RC	RC	RC	RC Staph	RC	RC Staph	RC
G. P.		Strep	Strep	Staph	Staph	Staph	Staph	RC	Staph	RC Staph

Subject 7

Axilla	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	RC
Groin	RC	Staph	RC	Staph	RC	Staph	Staph	RC	RC	RC
G. P.		Staph	Staph	RC	Staph	RC	RC	Staph	Staph	RC

Subject 8

Axilla	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph	Staph
Groin	Staph	Staph	RC	RC	Staph	Staph	Staph	RC	RC	RC
G. P.	RC	Staph	Staph	RC	Staph	RC	RC	Staph	RC	RC

TABLE 30

TYPES OF ORGANISMS FOUND IN HIGHEST DILUTION OF AEROBIC SAMPLE SHOWING GROWTH IN BUCCAL AND THROAT
(Microscopic Observation)

Subject 1

Body Area	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
Buccal	Strep	Strep	Staph	Strep Micro	Strep	Strep	Strep	Strep	Strep	Strep
Throat	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep

Subject 2

Buccal	Strep	Strep	Strep	Strep Micro	Strep	Staph	Strep	Strep	Strep	Strep
Throat	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep Staph

Subject 3

Buccal	Strep	Strep	Staph Strep	Strep Staph	Strep	Strep	Strep	Strep	Strep	Strep
Throat	Strep	Strep	Strep	Strep	Strep	Staph		Strep	Strep	Strep

Subject 4

Buccal	Strep			Strep	Rod	Strep	Strep	Strep	Strep	Strep
Throat	gram neg rod fusiform Strep	Strep RC	Strep	fusiform		Strep	Strep	Strep	Strep	Strep

Strep = Streptococcus; Staph = Staphylococcus; Micro = Micrococcus; RC = Rod corynebacterium

TABLE 30 (concluded)

TYPES OF ORGANISMS FOUND IN HIGHEST DILUTION OF AEROBIC SAMPLE SHOWING GROWTH IN BUCCAL AND THROAT
(Microscopic Observation)

Subject 5

Body Area	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
Buccal		Strep	Strep	Strep	Strep	Strep	Strep	Strep	long gm ± Rod Strep	Strep
Throat	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep

Subject 6

Buccal	Strep	Staph Strep	Strep	Strep Micro	Strep	Strep	Strep	Strep	Rod Strep	gm ± cocci Strep
Throat	Staph RC	Strep	Strep	Strep	Staph	Strep		Strep	Strep	Strep Staph

Subject 7

Buccal	Staph Micro	Strep	Strep	Strep	Strep	Strep		Strep	Strep	Strep
Throat	Strep	Strep RC	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep

Subject 8

Buccal	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Strep	Staph	Strep
Throat	Strep	Strep	Strep	RC	Strep	Strep	Strep	Staph	Strep	Staph

Strep = Streptococcus; Staph = Staphylococcus; Micro = Micrococcus; coryne = corynebacterium; RC = rod corynebacterium

TABLE 31

BACTERIA ISOLATED FROM SWABS OF EIGHT CHAMBER AREAS
(Microscopic observations)

Area	Sample Number				
	1	2	3	4	5
Water Faucet		Staphylococcus	Staphylococcus, Streptococcus	Staphylococcus Streptococcus	Staphyloccus
Toilet		Staphylococcus	Staphylococcus	Staphylococcus	
Transfer Lock Handle		Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Bed Post		Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Telephone	Streptococcus Staphylococcus	Staphylococcus		Staphylococcus	Staphylococcus
Floor Area	Gram neg. rod Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Chair	Staphylococcus Gram neg. rod	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus
Two Buttons		Staphylococcus	Staphylococcus Streptococcus		Staphylococcus Streptococcus

TABLE 31 (cont'd)

BACTERIA ISOLATED FROM SWABS OF EIGHT CHAMBER AREAS

(Microscopic observations)

Area	Sample Number				
	6	7	8	9	10
Water Faucet	Staphylococcus Streptococcus Gram neg. rod	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus Streptococcus Gram neg. rod	Staphylococcus
Toilet	Staphylococcus Streptococcus	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus Gram neg. rod	Staphylococcus Gram neg. rod
Transfer Lock Handle	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Bed Post	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus
Telephone	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus Streptococcus	Staphylococcus
Floor Area	Staphylococcus Streptococcus Gram neg. rod	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus
Chair	Staphylococcus	Staphylococcus Gram neg. rod	Staphylococcus	Staphylococcus	Staphylococcus
Two Buttons	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus

TABLE 31 (concluded)

BACTERIA ISOLATED FROM SWABS OF EIGHT CHAMBER AREAS

(microscopic observations)

Area	Sample Number			
	11	12	13	14
Water Faucet	Staphylococcus	Staphylococcus Streptococcus	Gram neg. rod Staphylococcus Streptococcus Long slender ± gram rods	Staphylococcus, Streptococcus Gram neg. rod
Toilet	Staphylococcus	Staphylococcus Gram neg. rod	Staphylococcus Gram neg. rod	Staphylococcus Gram neg. rod
Transfer Lock Handle	Staphylococcus	Staphylococcus	Staphylococcus, Streptococcus Gram-rods	Staphylococcus
Bed Post	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Telephone	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus Streptococcus
Floor Area	Staphylococcus	Staphylococcus	Staphylococcus, Streptococcus Long slender gram rods	Staphylococcus
Chair	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus Streptococcus
Two Buttons	Staphylococcus	Staphylococcus		Staphylococcus

TABLE 32

BACTERIA ISOLATED FROM SWABS OF EIGHT COTTAGE AREAS
(Microscopic observation)
Sample Number

Area	1	2	3	4	5
Filter					Gram positive rods, slender
Table Top	Gram neg. rods Diplococcus	Staphylococcus, Slender gram neg. rods	Staphylococcus	Staphylococcus	Short gram positive rods Streptococcus
Water Faucet	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus
Toilet		Gram neg. rod	Gram neg. rod Staphylococcus	Staphylococcus	Staphylococcus Gram neg. rod
Transfer Lock Handle		Staphylococcus	Staphylococcus	Staphylococcus, Streptococcus	Streptococcus
Bed Post		Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Floor Area	Diplococcus Gram neg. rod Lg. gram positive rods(Bacilli)		Slender gram negative rod	Staphylococcus	Staphylococcus
Chair	Slender gram negative rods Diplococcus	Staphylococcus Slender gram neg. rods	Slender gram negative rod	Staphylococcus	Staphylococcus

TABLE 32 (cont'd)

BACTERIA ISOLATED FROM SWABS OF EIGHT COTTAGE AREAS
(Microscopic observation)

Sample Number

Area	6	7	8	9	10
Filter	Staphylococcus Slender rod				
Table Top	Staphylococcus	Staphylococcus Slender gram neg. rod	Staphylococcus	Staphylococcus	Staphylococcus
Water Faucet	Diplococcus Streptococcus	Staphylococcus, Streptococcus	Staphylococcus	Staphylococcus	Staphylococcus
Toilet	Gram neg. rod, Staphylococcus	Staphylococcus, Diplococcus	Staphylococcus, Gram neg. rod		Staphylococcus
Transfer Lock Handle	Streptococcus	Staphylococcus, Streptococcus	Staphylococcus	Staphylococcus	Staphylococcus
Bed Post	Staphylococcus	Staphylococcus	Slender gram neg. rods	Staphylococcus	Staphylococcus
Floor Area	Gram neg. rods	Staphylococcus	Staphylococcus	Staphylococcus	
Chair	Slender gram positive rod	Staphylococcus	Staphylococcus Slender gram neg. rods	Staphylococcus Slender gram neg. rod	Staphylococcus Slender gram neg. rods

TABLE 32 (concluded)

BACTERIA ISOLATED FROM SWABS OF EIGHT COTTAGE AREAS

(Microscopic observation)

Area	Sample Number 11	12	13	14
Filter		Gram neg. rod, Bacillus		
Table Top	Staphylococcus	Staphylococcus Gram neg. rod	Gram neg. rod Staphylococcus	Staphylococcus
Water Faucet	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Toilet	Staphylococcus Gram neg. rod	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus
Transfer Lock Handle	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
Bed Post	Staphylococcus	Staphylococcus	Staphylococcus Gram pos. rods	Staphylococcus
Floor Area	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus, Slender gram neg.rod
Chair	Staphylococcus	Staphylococcus	Staphylococcus Streptococcus	Staphylococcus, slender gram neg. rod

TABLE 33

BACTERIA ISOLATED FROM WASH WATER
(Microscopic observation)

	Sampling Period					
	1	2		3	4	5
Subject	Aerobic	Aerobic	Anaerobic	Aerobic	Aerobic	Aerobic
1	GNR	Sarcina Staph	Staph	GNR	Staph	Staph, GNR
2	GNR	GNR		GNR	GNR NG Diplo Staph	Strep GNR Diplo
3	GNR	GNR, Diplo		Staph GNR	Staph GN	Staph Strep
4	GNR	Staph Sarcina		Staph Slender GPR GNR	GNR Long rod Staph	Diplo GNR Staph
5	GNR	Staph	Staph	Staph, GNR	RC Staph	GNR Staph
6	GNR	Staph		Staph Strep GNR	Staph	GNR Strep
7	Staph, RC	Staph	Staph	Staph	Staph Strep	
8	Staph, R	Staph		Staph Strep	Staph Strep	Staph GNR

Numbers in parenthesis indicate number of times isolated
Staph - Staphylococcus
Diplo = Diplococcus

Slender GPR = Slender gram positive rod
RC = Rod corynebacterium
FR = Fat rod

TABLE 34

BACTERIA ISOLATED FROM SWABS FROM NECK OF URINE BOTTLES
(Microscopic observation)

Subject	Sample Number			
	1	2	3	4
1	GNR Streptococcus	GNR Streptococcus	GNR	Staphylococcus
2	GNR	GNR	Staphylococcus	No Slide
3	GNR Streptococcus	Staphylococcus	Staphylococcus	GNR
4	GNR Streptococcus	Staphylococcus	Staphylococcus Streptococcus GNR	GNR
5	GNR	GNR	Staphylococcus	GNR
6	GNR	GNR	Staphylococcus Streptococcus	GNR
7	GNR Streptococcus	No Slide	No Slide	GNR
8	GNR Streptococcus	GNR Streptococcus	Staphylococcus	No Slide

GNR = Gram Negative Rods

TABLE 35

BACTERIA ISOLATED FROM THREE AREAS OF THE SUIT PRIOR TO DONNING

(Microscopic Observation)

Subject Number	Area		
	Crotch	Axilla	Foot
1	Staphylococcus	Staphylococcus Streptococcus RC	Staphylococcus Streptococcus RC
2	Streptobacillus Gram Negative Rod	Gram Negative Rod	Staphylococcus
3	Staphylococcus		Staphylococcus Streptococcus Gram Negative Rod
4	Gram Negative Rod	Staphylococcus Streptococcus	Staphylococcus
5	Staphylococcus Streptococcus	Staphylococcus	Staphylococcus
6	Gram Positive Diplococcus Staphylococcus Streptococcus Gram Negative Rod RC	Gram Negative Rod Staphylococcus	Staphylococcus Streptococcus Gram Negative Rod
7	Streptococcus Diplococcus Staphylococcus	Staphylococcus RC	Staphylococcus Streptococcus
8	Staphylococcus Streptococcus	RC Streptococcus	Streptococcus Gram Negative Rod Staphylococcus

RC - Rod Corynebacterium

TABLE 36

FOUR MOST FREQUENTLY OCCURRING AEROBIC BACTERIA
FOR EACH BODY AREA TESTED

Buccal	Throat	Axilla	Groin	G. P.
Streptococci	Streptococci	Micrococci including staphylococci	Micrococci including staphylococci	Micrococci including staphylococci
Micrococci including staphylococci	Micrococci including staphylococci	Corynebacteria	Corynebacteria	Corynebacteria
Corynebacteria	Lactobacilli	Aerobacter	Aerobacter	Streptococci
Lactobacilli	Corynebacteria	Actinomyces	Actinomyces	Sarcina

TABLE 37

PREDOMINATING ORGANISMS FOUND IN BODY AREAS
(Descending Order of Frequency of Isolation)

Body Area	Subject Number	1	2	3	4	5	6	7	8	9	10
Buccal	1		streptococci	streptococci	streptococci	streptococci	streptococci corynebacteria staphylococci	streptococci staphylococci	streptococci	streptococci corynebacteria	streptococci corynebacteria
	2		streptococci staphylococci	streptococci *staphylococci *corynebacteria	streptococci staphylococci gm neg rod	streptococci staphylococci	streptococci staphylococci	gm neg rod	streptococci	corynebacteria staphylococci	streptococci corynebacteria
	3	streptococci	streptococci	streptococci	*streptococci *staphylococci	streptococci staphylococci	streptococci	streptococci	streptococci	streptococci	streptococci staphylococci
	4	Str. salvarius staphylococci	streptococci staphylococci	streptococci staphylococci	*streptococci *corynebacteria staphylococci	streptococci staphylococci	staphylococci streptococci	streptococci	streptococci staphylococci	streptococci	streptococci corynebacteria staphylococci
	5		streptococci staphylococci	staphylococci	streptococci Haemophilus	streptococci	streptococci staphylococci	streptococci	streptococci staphylococci	*staphylococci *streptococci	staphylococci streptococci
	6	staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci		streptococci
	7	staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci
	8		streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci	streptococci staphylococci	streptococci staphylococci	streptococci
Throat	1	corynebacteria staphylococci streptococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci	streptococci corynebacteria staphylococci	staphylococci	streptococci corynebacteria	streptococci	gm neg rod
	2	staphylococci *Str. salvarius *corynebacteria gm neg rod neisseria	*streptococci *staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci
	3	neisseria streptococci	*streptococci *staphylococci corynebacteria	*streptococci *staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci corynebacteria	*streptococci *staphylococci	*streptococci *staphylococci	corynebacteria staphylococci streptococci	*streptococci *staphylococci
	4	streptococci corynebacteria	*streptococci *staphylococci corynebacteria	*streptococci *staphylococci	staphylococci streptococci	streptococci staphylococci corynebacteria	streptococci staphylococci	staphylococci streptococci	staphylococci streptococci	staphylococci corynebacteria streptococci	Str. mitis staphylococci
	5	streptococci staphylococci	*corynebacteria *staphylococci	*streptococci *staphylococci	streptococci staphylococci	staphylococci streptococci	*staphylococci *streptococci	staphylococci *streptococci	*streptococci staphylococci	streptococci staphylococci	streptococci staphylococci
	6	staphylococci streptococci	staphylococci streptococci	staphylococci streptococci	staphylococci	staphylococci streptococci	staphylococci streptococci	staphylococci	staphylococci	streptococci staphylococci gm neg rod	staphylococci
	7	gm neg rod	streptococci *neisseria *staphylococci	*staphylococci *streptococci	streptococci staphylococci	staphylococci streptococci	streptococci staphylococci	streptococci staphylococci	streptococci staphylococci	streptococci neisseria	streptococci staphylococci
	8	staphylococci streptococci	*streptococci *staphylococci	streptococci staphylococci	staphylococci streptococci	streptococci staphylococci	staphylococci	staphylococci streptococci	staphylococci streptococci	streptococci staphylococci	*streptococci *staphylococci
Axilla	1	corynebacteria staphylococci	*staphylococci *haemophilus *gm neg rod	corynebacteria	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci		staphylococci	corynebacteria	streptococci
	2	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci bacillus	staphylococci	staphylococci	staphylococci	staphylococci
	3	gm neg rod staphylococci	*staphylococci *corynebacteria gm neg rod	corynebacteria staphylococci gm neg rod	staphylococci corynebacteria gm neg rod	corynebacteria staphylococci	corynebacteria staphylococci gm neg rod	staphylococci corynebacteria	corynebacteria staphylococci	corynebacteria staphylococci gm neg rod	staphylococci corynebacteria
	4	staphylococci	staphylococci	*staphylococci *gm neg rod	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci
	5	staphylococci	staphylococci	staphylococci	staphylococci	corynebacteria staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	
	6	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	
	7	corynebacteria	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci gm positive rod	corynebacteria staphylococci	staphylococci	
	8	staphylococci	*corynebacteria *staphylococci	staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	*corynebacteria *staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	

TABLE 37

PREDOMINATING ORGANISMS FOUND IN BODY AREAS
(Descending Order of Frequency of Isolation)

Body Area	Subject Number	1	2	3	4	5	6	7	8	9	10
Groin	1	gm neg rod	*staphylococci *gm neg rod	staphylococci gm neg rod	staphylococci	staphylococci	staphylococci gm neg rod	staphylococci	staphylococci gm neg rod	gm pos rod	gm neg rod staphylococci corynebacteria
	2	staphylococci corynebacteria	staphylococci corynebacteria	staphylococci	staphylococci	staphylococci corynebacteria	corynebacteria staphylococci	staphylococci	staphylococci corynebacteria	staphylococci corynebacteria	staphylococci
	3	gm neg rod	staphylococci	staphylococci gm neg rod	staphylococci gm neg rod	staphylococci corynebacteria	staphylococci	corynebacteria staphylococci	staphylococci corynebacteria	staphylococci	*staphylococci *corynebacteria
	4	staphylococci	corynebacteria staphylococci	staphylococci	staphylococci	staphylococci	staphylococci corynebacteria	staphylococci	staphylococci	staphylococci	*staphylococci *corynebacteria
	5		*corynebacteria *staphylococci	staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci	staphylococci	corynebacteria staphylococci	staphylococci
	6		*corynebacteria *staphylococci	corynebacteria staphylococci	corynebacteria *staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	staphylococci corynebacteria
	7	staphylococci	corynebacteria staphylococci	staphylococci	*staphylococci *corynebacteria	*staphylococci *corynebacteria gm neg rod	corynebacteria staphylococci	staphylococci	corynebacteria *staphylococci	staphylococci	staphylococci
	8		*corynebacteria *staphylococci	*staphylococci	corynebacteria *staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci
Glans penis	1		staphylococci Entero	staphylococci corynebacteria	*staphylococci *corynebacteria	corynebacteria staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci	staphylococci corynebacteria	staphylococci corynebacteria gm neg rod
	2		staphylococci corynebacteria		staphylococci	staphylococci	corynebacteria staphylococci		staphylococci bacillus	staphylococci corynebacteria	staphylococci corynebacteria
	3		staphylococci		staphylococci	staphylococci	staphylococci corynebacteria	spreader	staphylococci	staphylococci	staphylococci
	4	corynebacteria staphylococci	staphylococci corynebacteria	staphylococci	staphylococci	staphylococci	staphylococci gm neg rod	staphylococci	staphylococci	staphylococci	staphylococci
	5		corynebacteria staphylococci		staphylococci	staphylococci	corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci
	6		corynebacteria staphylococci	staphylococci	staphylococci	streptococci staphylococci	neisseria	corynebacteria staphylococci	staphylococci	staphylococci	staphylococci
	7		corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	staphylococci	corynebacteria staphylococci	staphylococci	staphylococci	staphylococci	corynebacteria staphylococci
	8	staphylococci	corynebacteria staphylococci	corynebacteria staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	staphylococci	corynebacteria staphylococci	gm neg rod
Feces	1	*gm neg rod *corynebacteria	corynebacteria gm pos rod	gm neg rod corynebacteria	gm neg rod	gm neg rod	gm neg rod corynebacteria	gm neg rod	corynebacteria gm neg rod staphylococci	staphylococci gm neg rod	*gm neg rod *corynebacteria
	2	gm neg rod staphylococci corynebacteria	gm neg rod gm pos rod	gm neg rod	gm neg rod		gm neg rod	gm neg rod	gm neg rod	gm neg rod	gm neg rod
	3	gm neg rod staphylococci	gm neg rod				gm neg rod staphylococci	gm neg rod	gm neg rod		
	4	gm neg rod gm pos rod	gm neg rod corynebacteria	gm neg rod corynebacteria staphylococci	gm neg rod staphylococci streptococci	gm neg rod	gm neg rod corynebacteria		gm neg rod streptococci staphylococci	gm neg rod staphylococci	
	5		gm neg rod staphylococci gm neg rod	gm neg rod staphylococci	gm neg rod staphylococci	gm neg rod	gm neg rod	gm neg rod	gm neg rod	gm neg rod	gm neg rod
	6	staphylococci	streptococci gm neg rod staphylococci	streptococci	gm neg rod streptococci staphylococci	spreader	streptococci staphylococci gm neg rod	gm neg rod	gm neg rod streptococci	staphylococci gm neg rod	streptococci gm neg rod staphylococci
	7	gm neg rod	gm neg rod corynebacteria	corynebacter staphylococci	gm neg rod	gm neg rod staphylococci	gm neg rod staphylococci	staphylococci gm neg rod	gm neg rod staphylococci	staphylococci gm neg rod	gm neg rod
	8	gm neg rod corynebacteria staphylococci	*gm neg rod *streptococci neisseria	gm neg rod neisseria	gm neg rod	gm neg rod	gm neg rod staphylococci	gm neg rod	gm neg rod	gm neg rod	gm neg rod

TABLE 38

AEROBIC PLATE COUNTS FROM FECAL SAMPLES
(Millions)

Subject Number	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	2	46	55	1	tntc	23	33	30	8	110
2	8	19	200	170	200	200	50	39	120	44
3	4	10	n. s.	75	50	50	50	100	90	n. s.
4	40	8	5	2	12	4	0	1	1	n. s.
5	n. s.	137	240	56	50	60	200	100	100	125
6	50	90	28	50	tntc	350	0	1	4	16
7	15	150	70	15	10	30	3	17	3	36
8	8	22	18	16	13	25	40	50	75	1

6 zeros omitted
n. s. = no sample
tntc = to numerous to count

TABLE 39

HEIGHT OF GROWTH IN ANAEROBIC BROTH SERIAL DILUTION
(Billions)

Subject Number	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
1	100	1000	10000	1000	100	100	1000	10	10	100
2	10	100	100	100	1000	1000	1000	100	100	10
3	10	100	n.s.	1000	100	1000	10000	100	10	n.s.
4	10	1000	100	10000	100	1000	1000	100	100	n.s.
5	n.s.	10	100	100	1000	1000	100	100	10	10
6	1000	100	100	10000	100	1000	10	100	100	100
7	1000	1000	1000	1000	1000	100	100	10	100	100
8	10000	100	100	1000	1000	1000	100	100	100	100

9 zeros omitted
n.s. = no sample

TABLE 40

NUMBER OF TEN TIMES DIFFERENCE BETWEEN THE AEROBIC PLATE COUNT AND GROWTH IN THE ANAEROBIC SERIAL DILUTION IN FECAL SAMPLES

Subject Number	Sampling Period										
	1	2	3	4	5	6	7	8	9	10	Average
1	4	4	5	5	(tntc)	3	4	2	3	2	3.6
2	3	3	2	2	3	3	4	3	2	2	2.7
3	3	3	n.s.	4	3	4	4	2	2	n.s.	3.1
4	2	5	4	6	3	5	5	4	4	n.s.	4.2
5	n.s.	3	2	3	4	4	2	3	1	1	2.6
6	4	2	3	5	(tntc)	3	3	4	5	4	3.7
7	4	3	4	4	4	3	4	2	4	3	3.5
8	5	3	3	4	4	4	3	3	3	4	3.6
											3.4

n.s. = no sample
tntc = to numerous to count, not included in averages

TABLE 41

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 1

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
FA-1			1		1				4	
FA-2						1				
FA-3			2				2			
FA-4									1	
FA-5				1				1	1	
FA-6						1				
FA-7							1			1
FA-8										
FA-9										
FA-10										
FA-11				1	1		2			
FA-12										
FA-13										
FA-14	1									1
FA-15					3			4		
FA-16							2			
FA-17		4								
FA-18	2			2						
GD-1										
GD-2						2	1	1		
GD-3										
GD-4					2					
GD-5										
GD-6										
GD-7										1
Unkeyed			1							
TOTAL	3	4	4	4	7	4	8	6	6	3
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus	1					1				
Enterococci										
Miscellaneous										
TOTAL	1	0	0	0	0	1	0	0	0	0

TABLE 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 2

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
FA-1	1		2		2		2		2	
FA-2			1							
FA-3							2			
FA-4										
FA-5										
FA-6			1							
FA-7										
FA-8										
FA-9				1						
FA-10										
FA-11										
FA-12										
FA-13		1								
FA-14										
FA-15		2			1	1	2	2		1
FA-16										
FA-17			1							
FA-18									1	
GD-1										
GD-2										
GD-3										
GD-4					3					1
GD-5										
GD-6										1
GD-7										1
Unkeyed				2*						
TOTAL	1	3	5	3	6	1	6	2	3	4
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed Lactobacillus Enterococci Miscellaneous									1	
TOTAL	0	0	0	0	0	0	0	0	1	0

* Large gram positive cocci in chains that did not transplant

TABLE 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 3

Anaerobes	Sampling Period									
	1	2	3*	4	5	6	7	8	9	10*
FA-1				2			1		2	
FA-2				2						
FA-3	1				3	2				
FA-4										
FA-5					3				1	
FA-6										
FA-7										
FA-8										
FA-9										
FA-10									1	
FA-11										
FA-12										
FA-13	1	4			1					
FA-14										
FA-15				1						
FA-16										
FA-17				1		1		2		
FA-18										
GD-1										
GD-2										
GD-3							2			
GD-4										
GD-5						1				
GD-6										
GD-7										
Unkeyed						2		**1		
TOTAL	2	4	0	6	7	6	3	3	4	0
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus				1						
Enterococci										
Miscellaneous										
TOTAL	0	0	0	1	0	0	0	0	0	0

* No sample received

** Peptococcus

Table 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 4

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10*
FA-1				2	1	2	7	3	5	
FA-2										
FA-3	1						1			
FA-4										
FA-5										
FA-6										
FA-7										
FA-8										
FA-9										
FA-10										
FA-11										
FA-12	1			1		1				
FA-13										
FA-14				1	1					
FA-15										
FA-16										
FA-17										
FA-18	1	1		1	1	1	1	2		
GD-1										
GD-2										
GD-3										
GD-4										
GD-5										
GD-6										
GD-7										
Unkeyed						2				
TOTAL	3	1	0	5	3	6	9	5	5	0
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus			4							
Enterococci					1					
Miscellaneous										
TOTAL	0	0	4	0	1	0	0	0	0	0

* No sample received

Table 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 5

Anaerobes	Sampling Period									
	1*	2**	3	4	5	6	7	8	9	10
FA-1							1	2		
FA-2										
FA-3			1			1				
FA-4										
FA-5										
FA-6				1	2		2	1	1	2
FA-7										
FA-8										
FA-9			2	2						
FA-10										
FA-11										
FA-12										
FA-13										
FA-14			2						2	
FA-15										1
FA-16										
FA-17										
FA-18						2				
GD-1										
GD-2										
GD-3					2					
GD-4										
GD-5										
GD-6										
GD-7										
Unkeyed										
TOTAL	0	0	5	3	4	3	3	3	3	3
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus						1				
Enterococci										
Miscellaneous										
TOTAL	0	0	0	0	0	1	0	0	0	0

* No sample received
** No growth

Table 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 6

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
FA-1						1		1	2	
FA-2					2					
FA-3				2						
FA-4										
FA-5				1			1			2
FA-6										
FA-7					1					
FA-8	2									
FA-9										
FA-10				2						
FA-11										
FA-12										
FA-13										
FA-14					2			1		
FA-15		4	2	1	2	3		1	3	
FA-16										
FA-17										
FA-18										
GD-1										
GD-2										
GD-3							2			1
GD-4										
GD-5	2					1				
GD-6								2		
GD-7										
Unkeyed								1		
TOTAL	4	4	2	6	5	5	3	5	5	4
FN-1										
FN-2										
FN-3	1									
FN-4										
FN-5			1							
Unkeyed										
Lactobacillus										
Enterococci										
Miscellaneous										
TOTAL	1	0	1	0	0	0	0	0	0	0

Table 41 (cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 7

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
FA-1	2		1	1		4	2	1	2	1
FA-2										
FA-3										1
FA-4										
FA-5										
FA-6				1						
FA-7									1	
FA-8			1							
FA-9										
FA-10										
FA-11										
FA-12										
FA-13										
FA-14	2	1	2							
FA-15	1	2	1		5			7	2	3
FA-16										
FA-17										
FA-18	1	2								
GD-1										
GD-2										
GD-3										
GD-4										
GD-5	1									
GD-6										
GD-7										
Unkeyed				1	1					
TOTAL	7	5	5	3	7	4	2	8	5	5
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus										
Enterococci										
Miscellaneous										
TOTAL	0	0	0	0	0	0	0	0	0	0

Table 41 (concluded)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Subject 8

Anaerobes	Sampling Period									
	1	2	3	4	5	6	7	8	9	10
FA-1	1							1	1	
FA-2										
FA-3										
FA-4										
FA-5										
FA-6										
FA-7										
FA-8	2				2					
FA-9										
FA-10			1							
FA-11										
FA-12										2
FA-13		1		1		2				
FA-14	1	1		2					1	
FA-15	1	1		2					1	
FA-16										
FA-17						1				2
FA-18			1					1		
GD-1										
GD-2										
GD-3		1			2		3			
GD-4										
GD-5										
GD-6										
GD-7										
Unkeyed			2	1						
TOTAL	5	4	4	6	4	3	3	2	3	4
FN-1										
FN-2										
FN-3										
FN-4										
FN-5										
Unkeyed										
Lactobacillus								2		
Enterococci										
Miscellaneous										
TOTAL	0	0	0	0	0	0	0	2	0	0

TABLE 42

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY SUBJECT

Anaerobes	Subject Number							
	1	2	3	4	5	6	7	8
FA-1	6	9	5	20	3	4	17	3
FA-2	1	1	2			2		
FA-3	4	2	6	2	2	3	1	
FA-4	1							
FA-5	3		4			4		
FA-6	1	1			9		1	
FA-7	2					1	1	
FA-8						2	1	4
FA-9		1			4			
FA-10			1			2		1
FA-11								
FA-12	4			3			1	2
FA-13		1	6					4
FA-14	1			2	4	3	5	5
FA-15	8	9	1		1	13	21	5
FA-16	2							
FA-17	4	1	4					3
FA-18	4	1		8	2		3	2
GD-1								
GD-2								
GD-3	4	1	2		2	3		6
GD-4	2	3						
GD-5			1			3	1	
GD-6		1				2		
GD-7	1	1						
Unkeyed	1	2	3	2		1	2	3
TOTAL	49	34	35	37	27	43	51	38
FN-1								
FN-2								
FN-3						1		
FN-4								
FN-5						1		
Unkeyed			1					
Lactobacillus	2			4	1			2
Enterococci		1		1				
Miscellaneous								
TOTAL	2	1	1	5	1	2	0	2

TABLE 43

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY PERIOD

Anaerobes	Sampling Period										
	1*	2	3*	4	5	6	7	8	9	10*	Total
FA-1	4		4	5	4	7	13	8	18	1	64
FA-2			1	2	2	1					6
FA-3	2		3	2	3	3	5			2	20
FA-4									1		1
FA-5				2	3		1	1	2	2	11
FA-6			1	2	2	1	2	1	1	2	12
FA-7					1		1		1	1	4
FA-8	4		1		2						7
FA-9			2	3							5
FA-10			1	2					1		4
FA-11											
FA-12	1			2	2	1	2			2	10
FA-13	1	6		1	1	2					11
FA-14	4	2	4	3	3			1	3		20
FA-15	2	9	3	4	9	4	2	13	6	6	58
FA-16							2				2
FA-17		4	1	1		2		2		2	12
FA-18	4	3	1	3	1	3	1	3	1		20
GD-1											
GD-2											
GD-3		1			4	2	8	1		2	18
GD-4					5						5
GD-5	3					2					5
GD-6								2		1	3
GD-7										2	2
Unkeyed			3	4**	1	4		2***		2	14
TOTAL	25	25	25	36	43	32	37	34	34	2 3	314
FN-1											
FN-2											
FN-3	1										1
FN-4											
FN-5			1								1
Unkeyed				1							1
Lactobacillus	1		4			2		2			9
Enterococci					1				1		2
Miscellaneous											
TOTAL	2	0	5	1	1	2	0	2	1	0	14

* Some samples not received during this period
** Large gram positive cocci in chains that did not transplant
*** Peptococcus (one)

TABLE 44

COMPARISON OF FREQUENCY OF OCCURRENCE OF FA TYPES IN THIS STUDY AND NASA STUDY

Anaerobe by Frequency	Total Occurrence	Frequency of Occurrence on NASA Study
FA-1	64	1
FA-15	58	2
FA-3	20	3
FA-14	20	7
FA-18	20	10
FA-6	12	6
FA-17	12	11
FA-5	11	4
FA-13	11	17
FA-12	10	5
FA-8	7	8
FA-2	6	12
FA-9	5	16
FA-7	4	15
FA-10	4	9
FA-16	2	13
FA-4	1	18
FA-11	0	14

TABLE 45

OCCURRENCE OF STRICT ANAEROBES IN GLANS PENIS AND GROIN SAMPLES

Sampling Period	Peptococcus 1	Peptococcus 2	Miscellaneous
1		3, 6(2x), 4*	
2	5d	8	
3		3, 8	Eubacterium-5
4			
5	2c, 5d	8e, 3, 5e	Veillonella-3
6	8	1a	
7	2ad, 2, 5ad	8ae, 8e, 3c, 3e, 2ac	
8	5ad, 7a, 8	2a, 8e(3x), 3a, 4(2x)	Veillonella-1, 3
9	1, 5	1b, 8	Eubacterium-6, 4, 3
10	2, 7ad	2e	Eubacterium-6

* Groin only
Numbers in table refer to subject number
Numbers in parenthesis indicate number of times cultures were isolated

Peptococcus 1 - Gram positive cocci, singly in pairs, tetrads and masses, very anaerobic with gas, usually reduced litmus only, slight with granular or floccular sediment.

Peptococcus 2 - Gram positive cocci, singly in pairs and masses, very anaerobic, no gas, reduced litmus, clear to moderate with granular or floccular sediment, all sugars, pH usually about neutral, (growth on sides).

(a) = Gelatin liquefaction
(b) = Slight acid
(c) = Dark sediment
(d) = ARC with proteolysis
(e) - Alkaline pH

TABLE 46

OCCURRENCE OF STRICT ANAEROBES IN THROAT AND BUCCAL AREA SAMPLES

Sampling Period	Area	Peptococcus 2	Veillonella	Fusobacterium	Miscellaneous
1	Throat				FA3-6; Unident. -5; Clostridium acidiurici-1
	Buccal		7,5,6		FA8-7; Lactobacillus-2(2x); Sphaerophorus-1
2	Throat	6,2			FA18-4
	Buccal	5,3			
3	Throat	3b			
	Buccal	6,3	1,8	6	
4	Throat			4	
	Buccal		1,2,4,8		Unident. fac. anaer. -5
5	Throat	4b	1,3,4,5,6,7,8		Lactobacillus-3
	Buccal		1,3,5,6,7		
6	Throat		2,5,7	5	PS_1-5
	Buccal	8	2,8,5,6,7	5	
7	Throat	2b	6		
	Buccal	5b	5,7		Lactobacillus-1
8	Throat	6b			
	Buccal	4	2,6,7	7	
9	Throat			5	Bacteroides unicates-7
	Buccal		1,7,5		
10	Throat	4b			Catenabacter-3; Lactobacillus-3
	Buccal	1b, 5b			Bacteroides-3; Lactobacillus-6

TABLE 47

LIST OF PRIMARY CULTURE MEDIA FOR EACH BODY AREA
AEROBIC AND ANAEROBIC

	Eye	Throat	Buccal	Axilla	Groin	G. P.	Feces
			Aerobic Samples				
Actinomycete Agar	x	x	x	x	x	x	x
Blood Agar (2)	x	x	x	x	x	x	x
Desoxycholate Agar	x	x	x				
PPLO Agar	x	x	x	x	x	x	x
Fungi Media Agar		x	x	x	x	x	x
Mitis Salivarius Agar		x	x	x	x	x	x
MacConkey's Agar				x	x	x	x
			Anaerobic Samples				
Anaerobic Blood Agar	x	x	x	x	x	x	x
Chocolate Agar	x	x	x			x	
Rogosa's Agar		x	x				x
Deep Blood Agar Tubes			x				
Dilution Series	1-4	1-6	1-6	1-4	1-5	1-4	1-12*
Agar Shakes	x	x	x	x	x	x	x**
Brewer Plates		x	x			x	x**
Counting Plates							x**

* Gall's Broth
** Gall's Agar

TABLE 48

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA (OBLIGATE)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk **	Gelatin	pH
FA-1	slender gram + rod singly and in chains; distinct rods uniformly spaced	very fine colonies; very anaerobic	heavy turbidity with slime developing	4+ 4+ slimy sediment	4+ 4+ slimy sediment	4+ 4+ slimy sediment	2+ 2+ slight slime	+ 2+	delayed Arc with proteolysis	no liquefaction	7.0
FA-2	slender gram + rod in chains, with tadpole formation	diffuse colonies very anaerobic	heavy with slime	4+ with silky turbidity 4+ slime	3+ with silky turbidity 3+ slime	3+ with silky turbidity 3+ slime	± +	± ±	delayed Arc with proteolysis	no liquefaction	6.4
FA-3	medium to small gram negative elongate pointed rods in pairs	diffuse growth; heavy gas; very anaerobic	heavy with slimy sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	delayed Arc with proteolysis and gas	no liquefaction	7.5
FA-4	slender gram positive, sometimes slightly curved rod, singly	small colonies; very anaerobic	moderate turbidity	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	2+ sediment 2+ sediment	2+ sediment 2+ sediment	Arc strong; delayed proteolysis	no liquefaction	5.6

* Results obtained under NASA Contract NASw-738

** Acid reduced curd

TABLE 48 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-5	short, medium slightly curved gram positive rod, singly; often developing clusters	medium colonies, very anaerobic	moderate turbidity	4+ slime 4+ slime	4+ slime 4+ sediment	4+ slime 4+ sediment	4+ slime 4+ slime	± + -	delayed Arc** with proteolysis	no liquefaction	5.5-5.8
FA-6	gram positive medium rods, tending to form clusters some slightly curved	medium colonies, very anaerobic	clear slimy sediment	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	+ slight slime + slight slime	Arc	no liquefaction	6.6
FA-7	small gram negative slender rod tendency towards bipolar staining	fine colonies; very anaerobic	moderate turbidity slime	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	+ + slime	+ +	Arc delayed proteolysis	no liquefaction	6.6
FA-8	tiny gram negative slender rods, slightly curved	fine colonies; very anaerobic	clear with sediment	+ 3+	+ 3+	+ 3+	+ 3+	+ 3+	partial reduction orange color	no liquefaction	6.9

* Results obtained under NASA Contract NASw-738
** Acid reduced curd

TABLE 48 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-9	medium to large pleomorphic gram positive rod in pairs and short chains; chain has characteristic hooked or loop shape - older cultures form heavy gram positive aggregation	haze; very anaerobic	moderate turbidity	3+ slight slime 3+ moderate slime	3+ slight slime 3+ moderate slime	+ slime 3+ slime	± slime + slight slime	clear with slight slime +	delayed Arc** with ± proteolysis	no liquefaction	7.0
FA-10	very small gram positive rods in chains with a tendency for bipolar staining, sometimes slightly pointed	fine colonies very anaerobic	heavy with floccular sediment	4+ fluffy sediment 4+ sediment	4+ fluffy sediment 4+ sediment	4+ fluffy sediment 4+ sediment	3+ 4+ sediment	+ sediment 4+ sediment	delayed Arc with proteolysis	no liquefaction	6.7
FA-11	medium short gram positive rods, some slightly curved, older cultures tend toward gram positive aggregation	fine colonies very anaerobic	heavy turbidity	3+ 3+ sediment	3+ 3+ sediment	3+ sediment 3+ sediment	3+ 3+ sediment	± sediment clear with slight sediment	Arc with proteolysis	no liquefaction	6.5

* Results obtained under NASA Contract NASw-738

** Acid reduced curd

TABLE 48 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-12	gram positive tiny pointed rods in chains with many coccoid forms	medium colonies very anaerobic with slight gas	heavy with slime	3+ slime 3+ slime	3+ slime 3+ slime	+ with slime 3+ slime	± slime + slime	± slime ± slime	delayed Arc** with proteolysis	no liquefaction	7.2
FA-13	small gram negative cocci in masses	fine colonies; heavy gas; very anaerobic	moderate turbidity	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	R***	no liquefaction	6.7
FA-14	gram negative rods long slender with gram positive areas	tiny colonies very anaerobic with heavy gas	heavy turbidity gas	4+ slight slime gas 4+	4+ slight slime 4+	+ 3+ sediment	± 3+ slime	± 3+ slime	R, whey carmelization	no liquefaction	6.75
FA-15	short fat gram negative rod, singly and in pairs; some with pointed ends	delayed haze; heavy gas; very anaerobic	heavy with slight slime	4+ slight slime 4+ slight slime	4+ slight slime 4+ slight slime	+ 4+ black slime	2+ slight slime 4+ slime	± ±	delayed Arc with whey	no liquefaction grey sediment	6.7

* Results obtained under NASA Contract NASw-738
** Acid reduced curd
*** Reduced

TABLE 48 (cont'd)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-16	gram positive pleo rods; some curved and some tadpole forms	haze with anaerobic collar	heavy with slime	+ curly slime 3+ slime	+ curly slime 3+ slime	+ curly slime 3+ slime	clear slime + slime	-	ARC**	no liquefaction	6.8
FA-17*	large gram positive rod singly and in pairs forming palisades and V's	fine colonies very anaerobic slight gas, occasionally	slight with finely granular sediment and side growth	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	ARC with proteolysis	no liquefaction	6.6
FA-18	gram positive long slender rods, irregular staining	fine colonies very anaerobic	slight with slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	ARC delayed	no liquefaction	6.3 to 6.6

*First week readings were the same as the 24 hour readings.

TABLE 48 (cont'd)

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA (FACULATIVE)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FN-1	gram positive pointed rods in pairs and short chains	fine colonies facultative anaerobic	heavy with slime	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	delayed ARC	no liquefaction	6.7
FN-2	gram positive coccobacillus pairs and chains	medium colonies facultative anaerobic	clear with growth on sides and white sediment	3+ granular sediment 3+ granular sediment	3+ granular sediment 3+ granular sediment	3+ granular sediment 3+ granular sediment	+ granular sediment 3+ granular sediment	± + with sediment	ARC with proteolysis	no liquefaction	6.5
FN-3	small round cocci in short chains becoming less discrete with age	discrete colonies with heavy gas facultative anaerobic	moderate with white sediment	3+ granular sedi- 4+ granular sediment	3+ granular sediment 4+ granular sediment	4+ sediment 4+ granular sediment	3+ 3+ granular sediment	± ±	ARC with proteolysis	no liquefaction	6.4
FN-4	gram positive elongate cocci in short chains	fine colonies faculative anaerobic	moderate	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	delayed soft ARC	no liquefaction	6.5

TABLE 48 (cont'd)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FN-5	gram positive diplococci in pairs and short chains;	fine colonies faculative anaerobic	moderate with floccular sediment	3+ floccular sediment 4+ floccular sediment	3+ floccular sediment 4+ floccular sediment	3+ floccular sediment 4+ floccular sediment	3+ floccular sediment 4+ floccular sediment	+ sediment + sediment	ARC with slight proteolysis	no liquefaction	7.3 to

TABLE 48 (cont'd)

SEVEN NEW TYPES OF OBLIGATE ANAEROBES (SPACE DIET - GD SERIES)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
G.D. 1	short gram negative rod in pairs and chains, some pointed	fine colonies heavy gas very anaerobic	heavy floccular sediment	4+ with slime 4+ with black slime	4+ with slime 4+ with black slime	4+ with slime 4+ with black slime	2+ with slime 4+ with black slime	1+ with slime 4+ with black slime	delayed Arc* with proteolysis	black bottom no liquefaction	6.7
G.D. 2	gram negative short rod in pairs	small colonies very anaerobic	moderate with floccular slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	3+ with floccular + slight floccular slime	Arc with proteolysis	no liquefaction	6.2 6.4
G.D. 3	gram negative pointed rods	tiny colonies very anaerobic	moderate with moderate black sediment sometimes fluffy	2+ with slime 3+ with slime sometimes dark	2+ with slime 3+ with slime sometimes dark	2+ with slime 3+ with slime	2+ with slime 3+ with slime	2+ with slime 3+ with slime	reduced	no liquefaction	6.8

* Acid reduced curd

** Results obtained under contract AF33(615)-1748, "Determination of Aerobic and Anaerobic Microflora of Human Feces

TABLE 48 (cont'd)

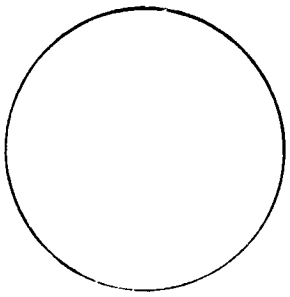
Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litnus Milk	Gelatin	pH
G.D. 4	gram negative slender rods in pairs some pleomorphic	tiny colonies heavy gas very anaerobic	moderate with granular sediment sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	3+ with slime and gas 3+ with slime sometimes dark	delayed Arc* with slight proteolysis	no liquefaction	6.3 6.4
G.D. 5 and G.D. 5a	gram ± medium rods in short chains	small colonies very anaerobic	clear to moderate with balls of sediment	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	2+ with granular sediment 3+ with slime or granular sediment sometimes black	Arc with proteolysis	no liquefaction	6.6**

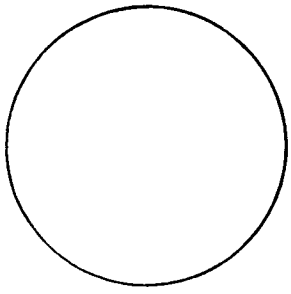
* Acid reduced curd
** G.D. 5a pH 6.2 to 6.4

TABLE 48 (concluded)

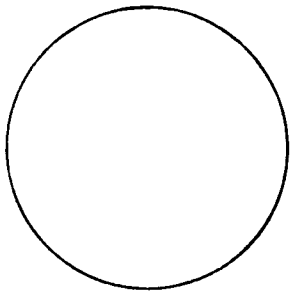
Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
G.D. 6	gram negative short pleomorphic rods singly and in pairs	tiny colonies heavy gas very anaerobic	slight to moderate with slimy sediment	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	+ with slimy sediment 3+ with brown slime	delayed Arc* with proteolysis	no liquefaction	5.9
G.D. 7	gram ± short pleomorphic rods in pairs some pointed	tiny colonies heavy gas very anaerobic	4+ with dark slime	4+ with slime and heavy gas 4+ with heavy black slime	4+ with slime and heavy gas 4+ with heavy black slime	4+ with slime and heavy gas 4+ with heavy black slime	3+ with heavy slime and gas 4+ with heavy black slime	3+ with heavy slime and gas 4+ with heavy black slime	reduced	no liquefaction black bottom	6.8

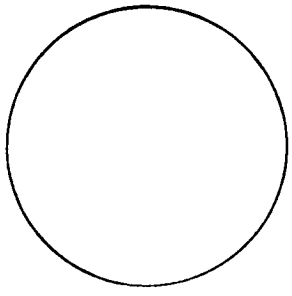
* Acid reduced curd



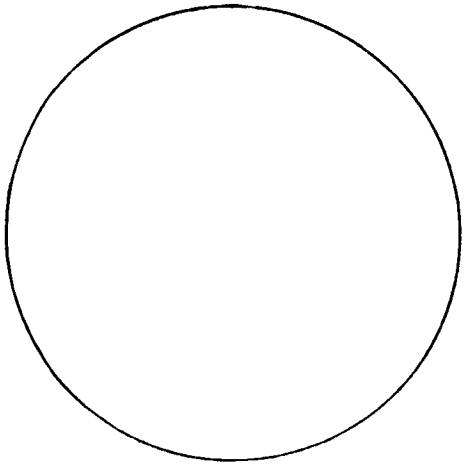


Petri plates with MacConkey's agar showing typical colonies of E. coli (Subjects 1 and 4 initial sampling period).

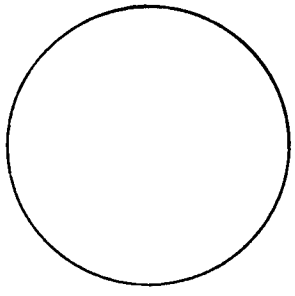


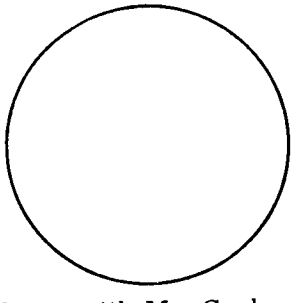


Petri plates with MacConkey's agar showing many colonies of Bethesda-Ballerup (Top - Subject 6 initial sampling period; Bottom -- Subject 4 second sampling period).



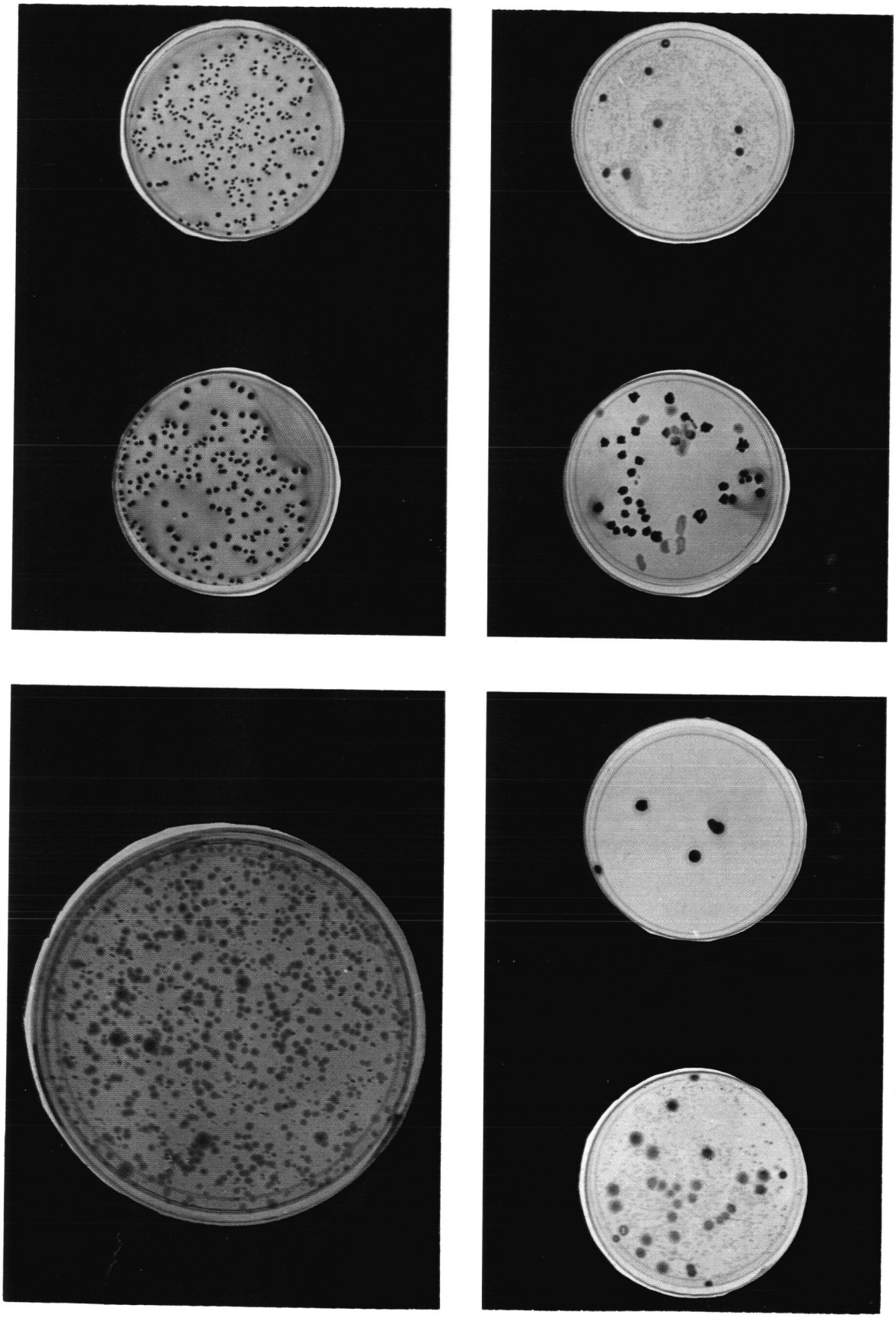
Petri plate with Gall's agar inoculated with 1.0 ml of 1/8000 dilution of wash water from "space sink" shared by Subjects 1 and 4 showing innumerable colonies.

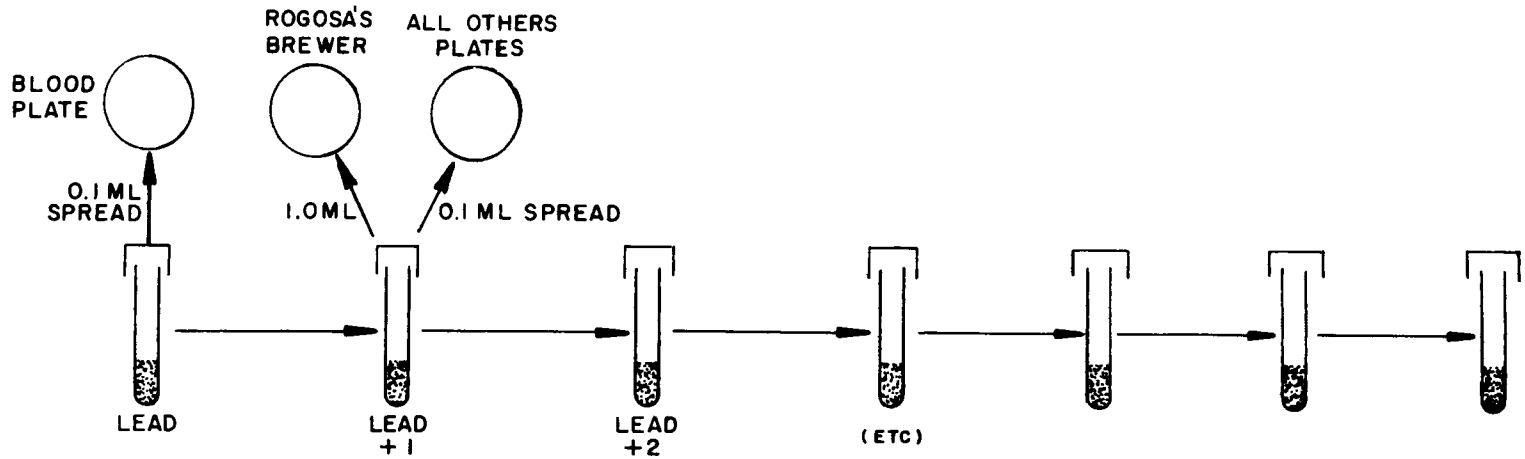




Petri plates with MacConkey's agar, Top from Subject 4 in 4th sampling period showing E. coli; Bottom from Subject 6, 7th sampling period showing Bethesda-Ballerup.

Figure 1. Bacterial Colonies from Feces and Wash Water of Subjects 1 and 4.





Plates made from Lead tube + 1 listed for each body area in Table 47.

All plates except fungi incubated at 37°C. Fungi incubated at room temperature.

Sample No. 1, 2, 3 - amount transferred - 1 ml
Sample No. 4 - amount transferred - 0.5 ml
Sample No. 5 and thereafter - amount transferred 0.5 ml first tube;
0.25 ml thereafter.

Number of tubes in culture series:

Eye	1 - 4	Buccal	1 - 6	Groin	1 - 5
Throat	1 - 7	Axilla	1 - 4	G. P.	1 - 4

Figure 2. Aerobic or Anaerobic Cultural Series for All Body Areas

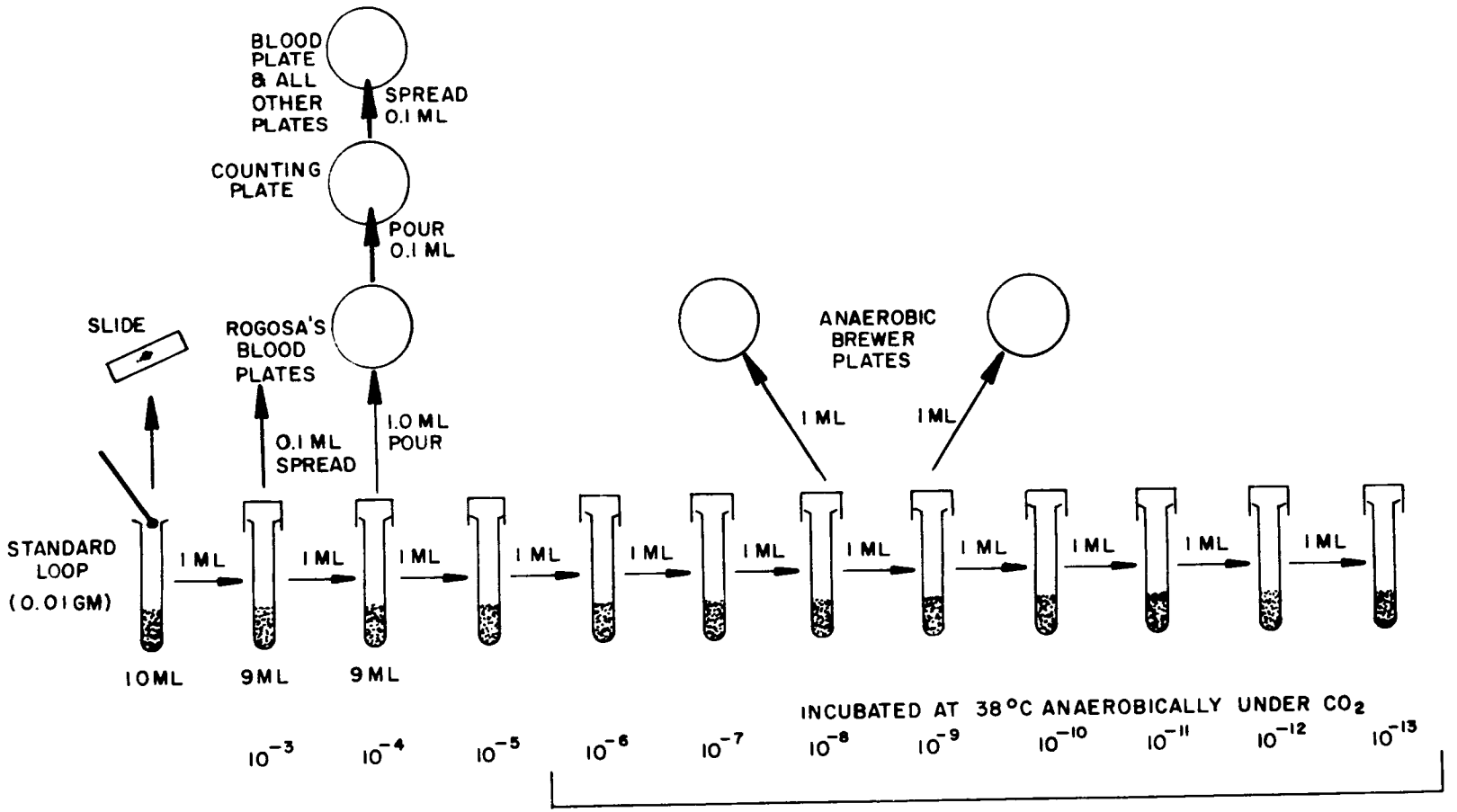
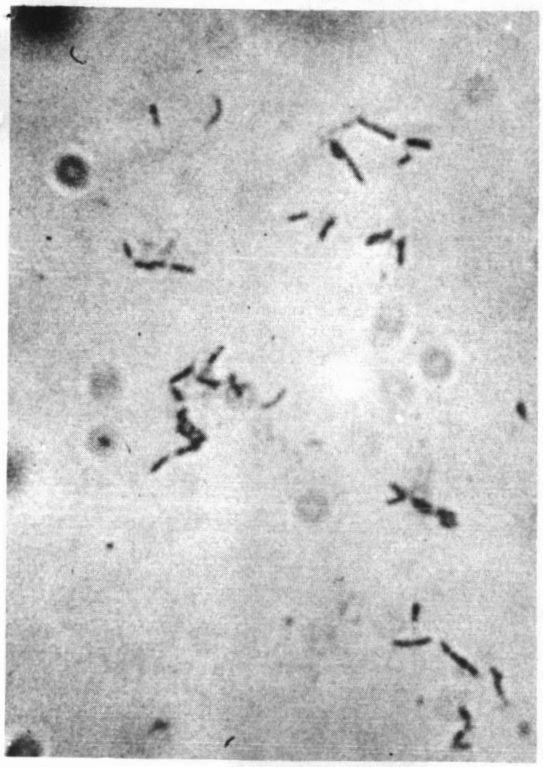
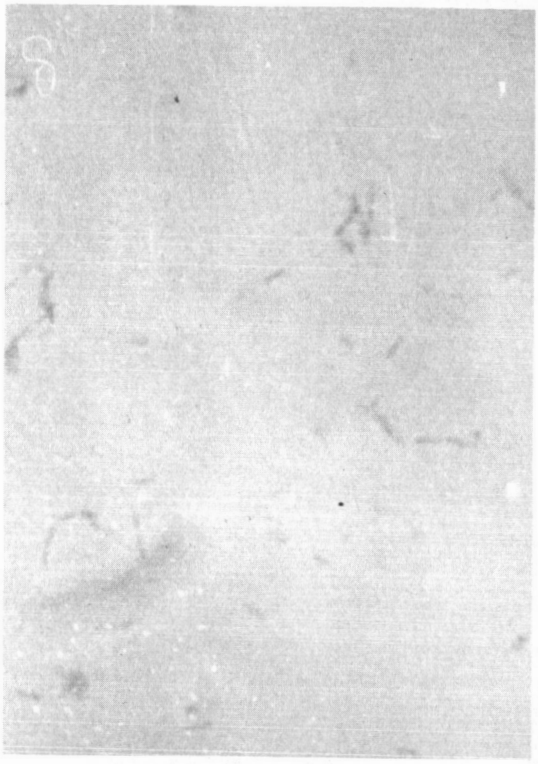


Figure 3. Anaerobic Dilution Series (Feces)

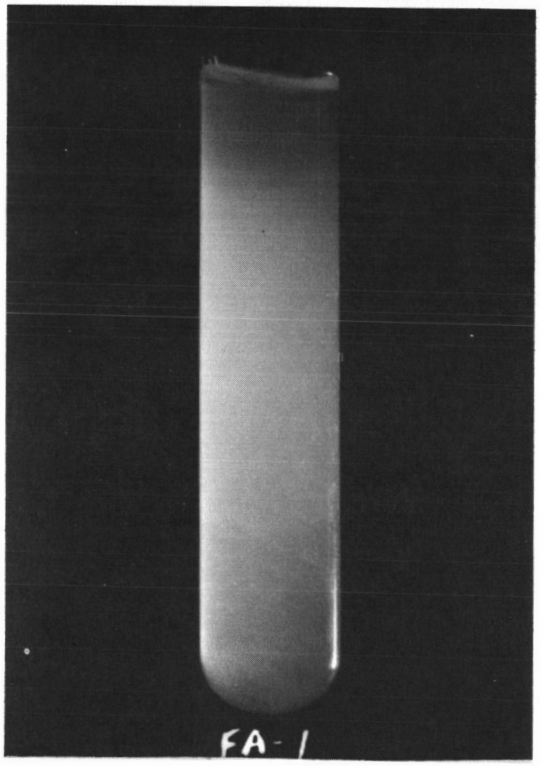


24 Hours (X8000)



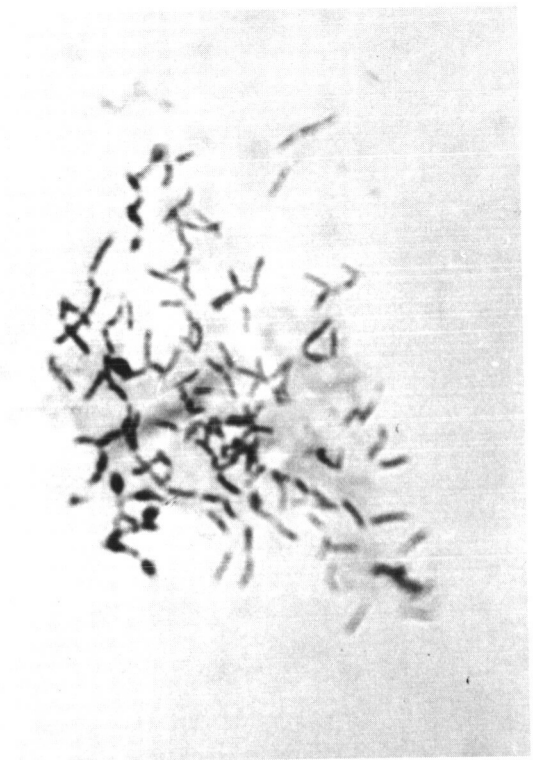
48 Hours (X8000)

1 Week (X8000)



Agar Shake

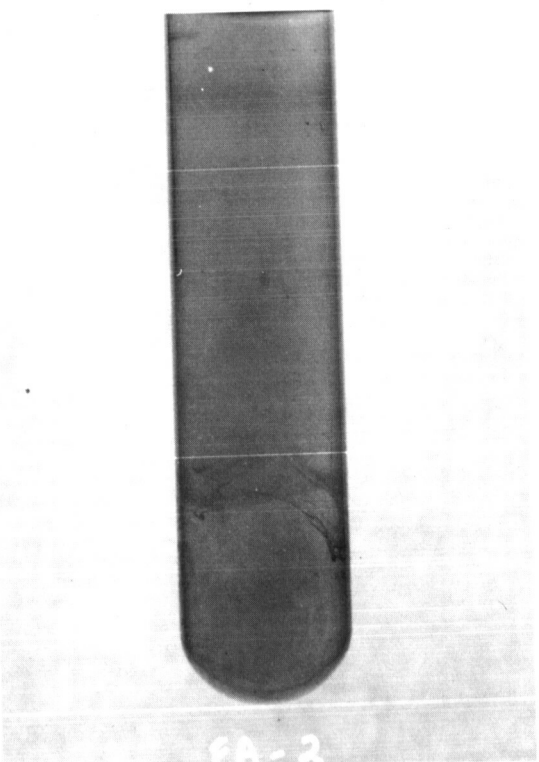
Figure 4. Pictures of Type Cultures



24 Hours (X8000)

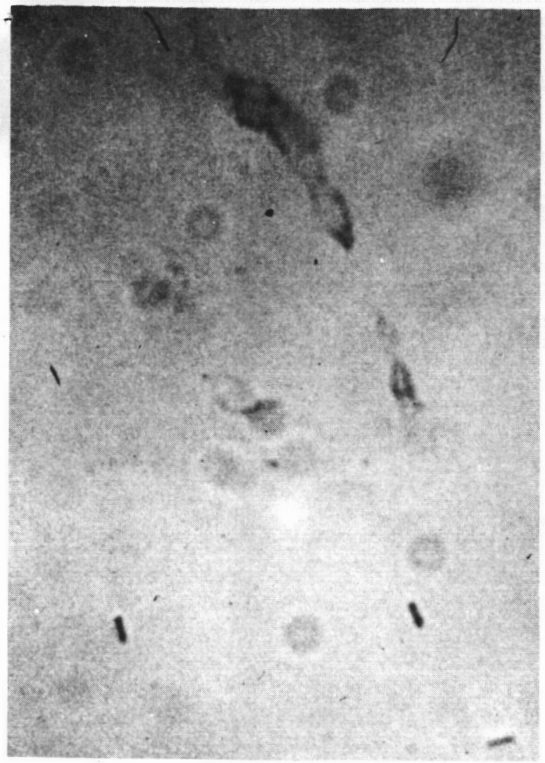
48 Hours (X8000)

1 Week (X8000)



Agar Shake

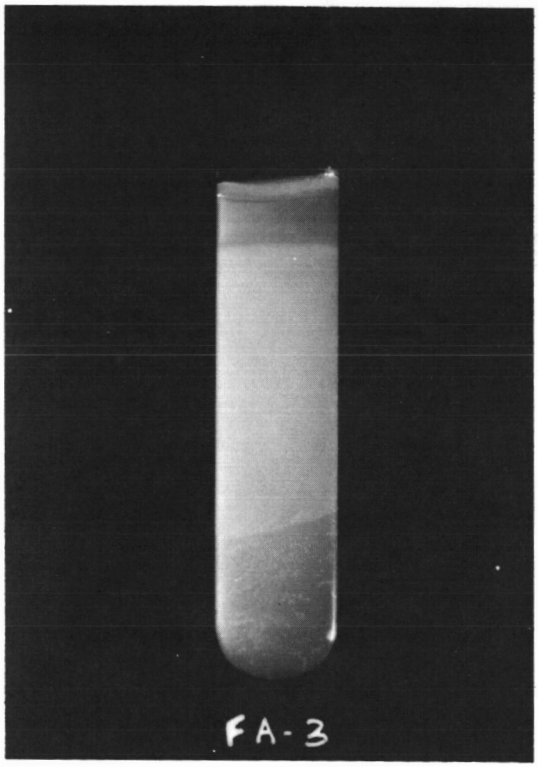
Figure 4. Continued



24 Hours (X8000)

48 Hours (X8000)

1 Week (X8000)

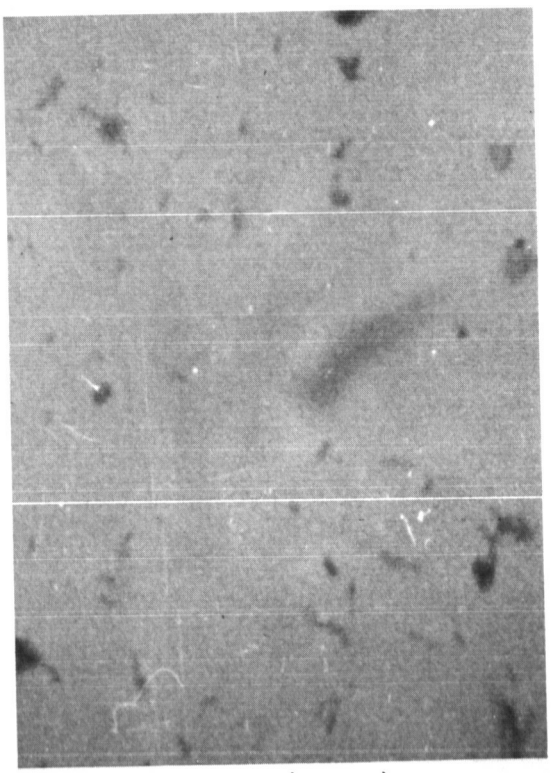


Agar Shake

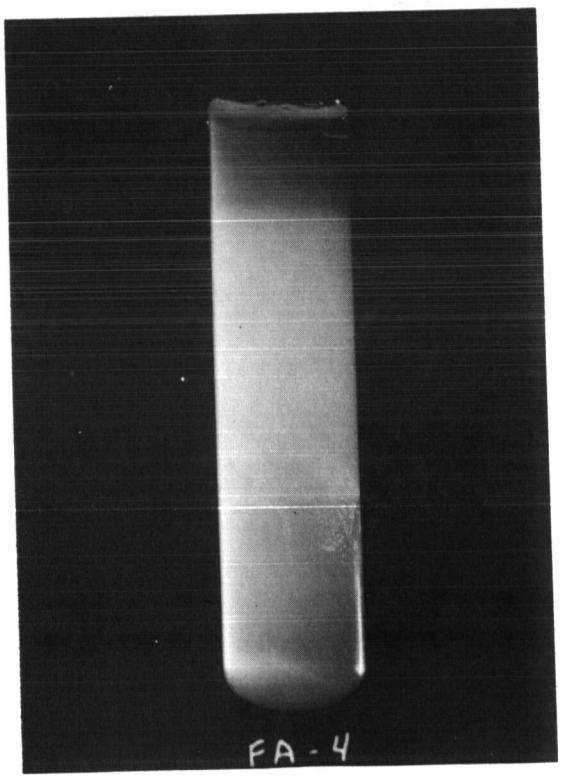
Figure 4. Continued

24 Hours (X8000)

48 Hours (X8000)

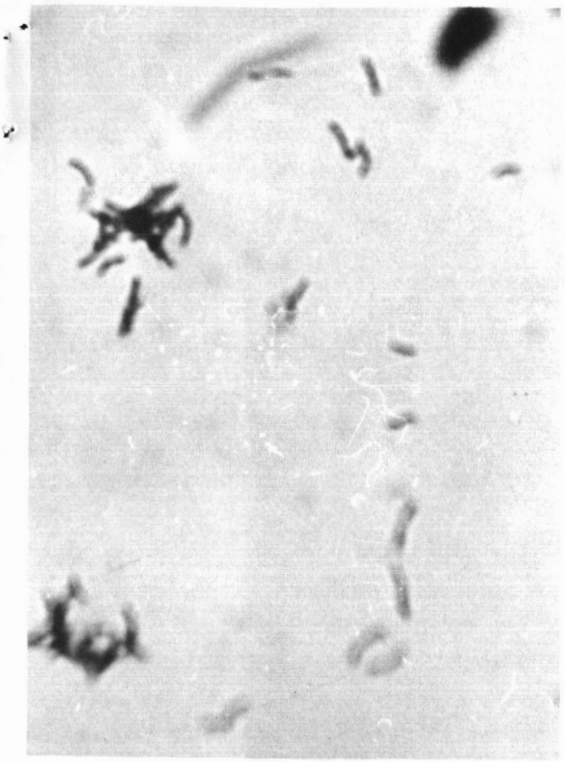


1 Week (X8000)

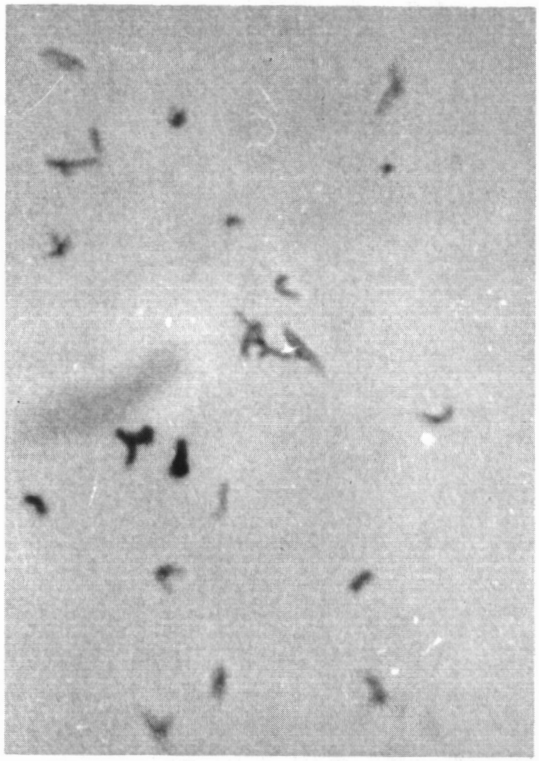


Agar Shake

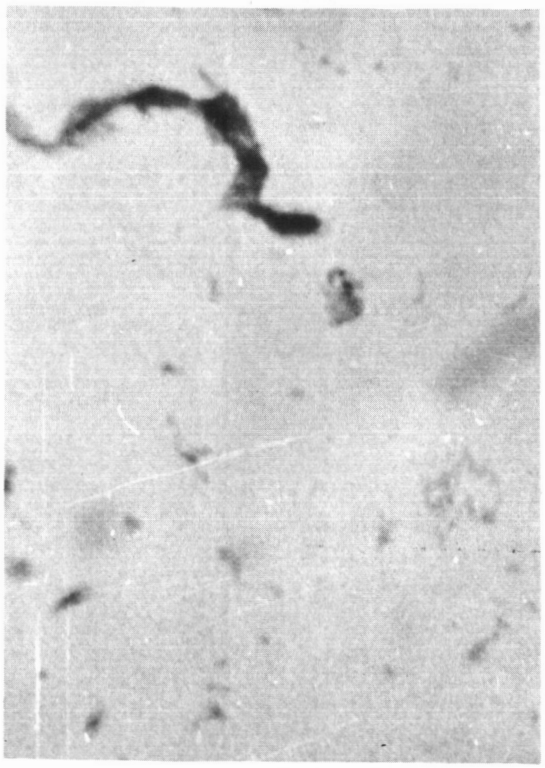
Figure 4. Continued



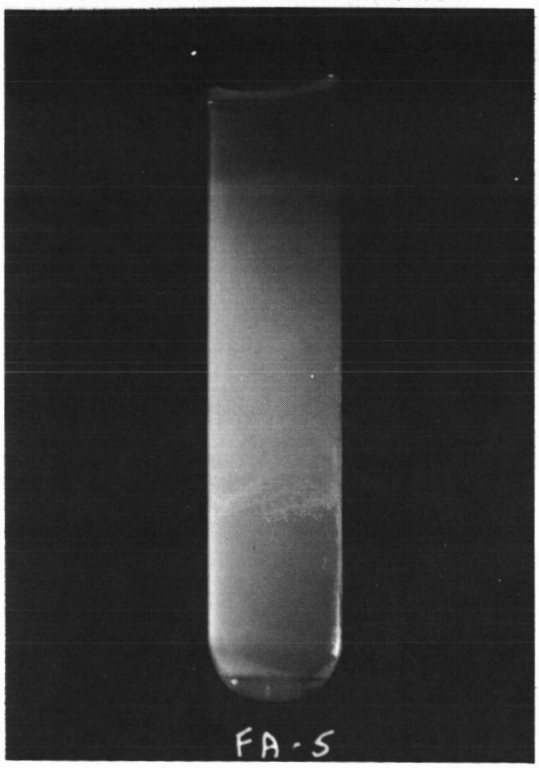
24 Hours (X8000)



48 Hours (X8000)



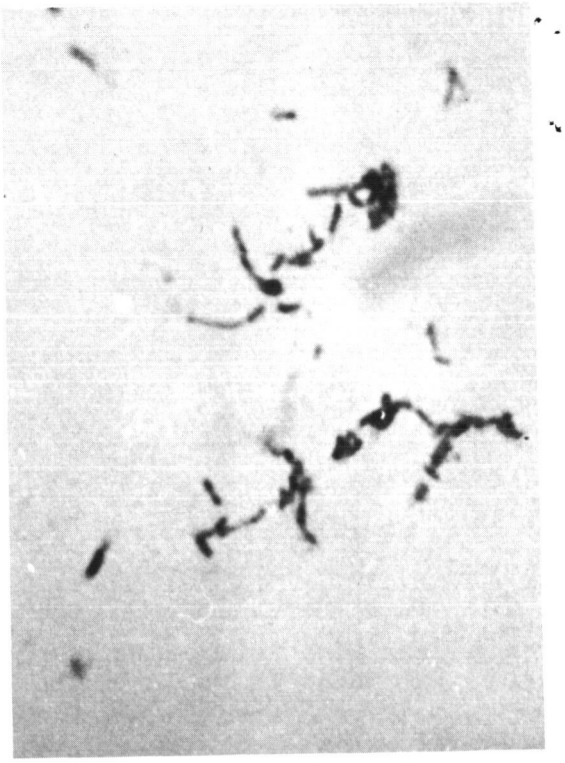
1 Week (X8000)



Agar Shake

Figure 4. Continued

24 Hours (X8000)



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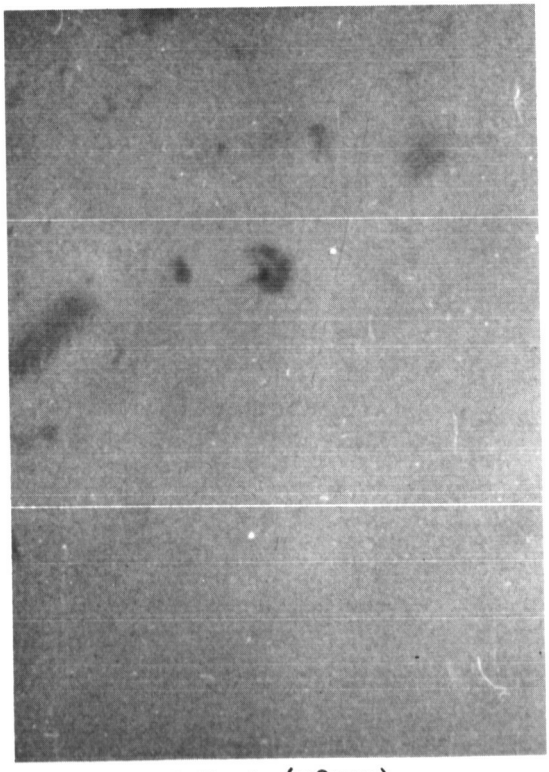
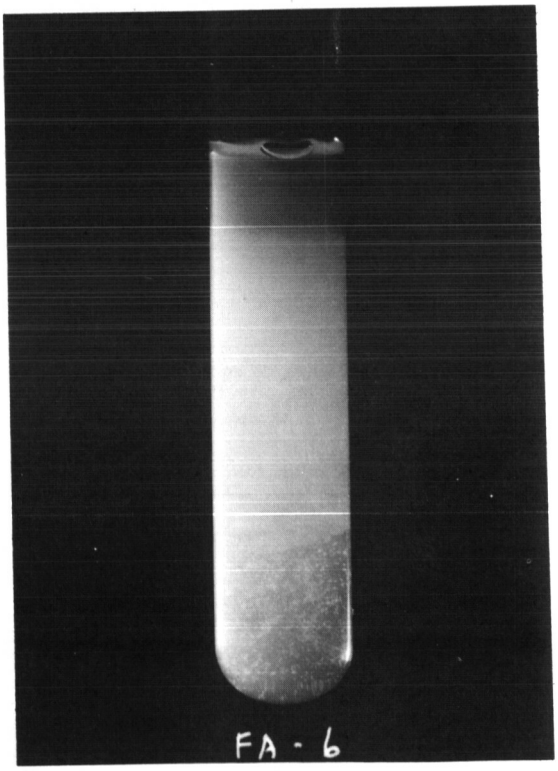


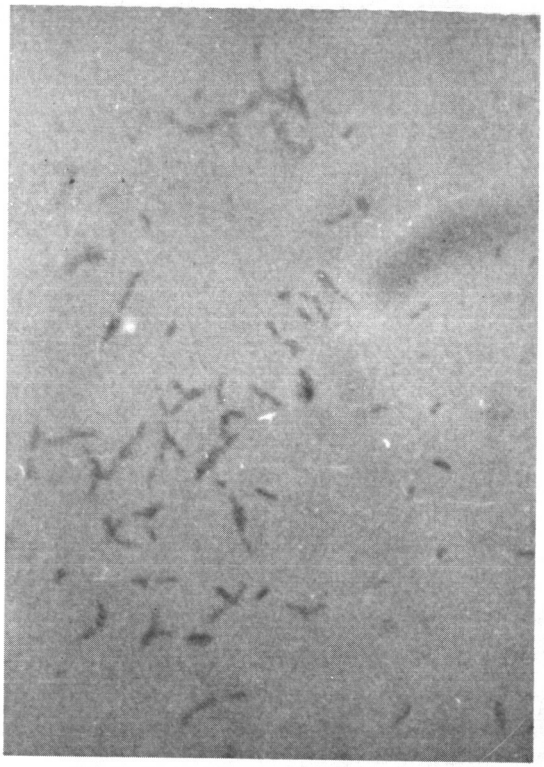
1 Week (X8000)



Agar Shake

Figure 4. Continued

24 Hours (X8000)



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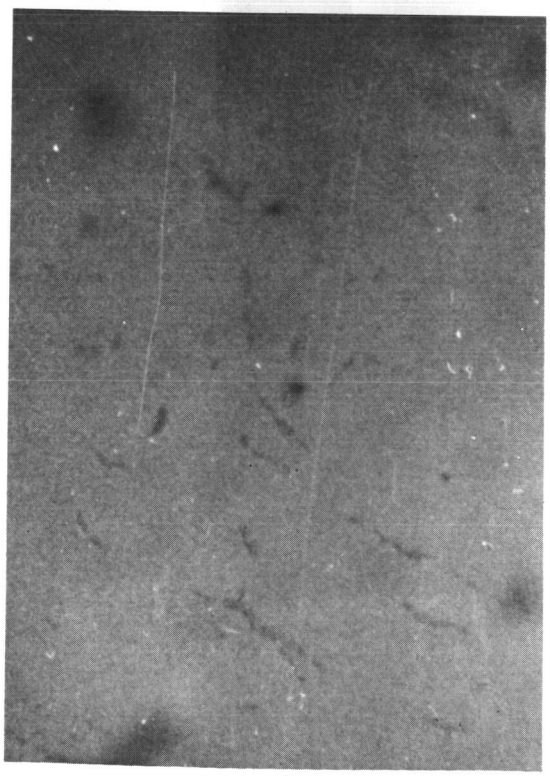
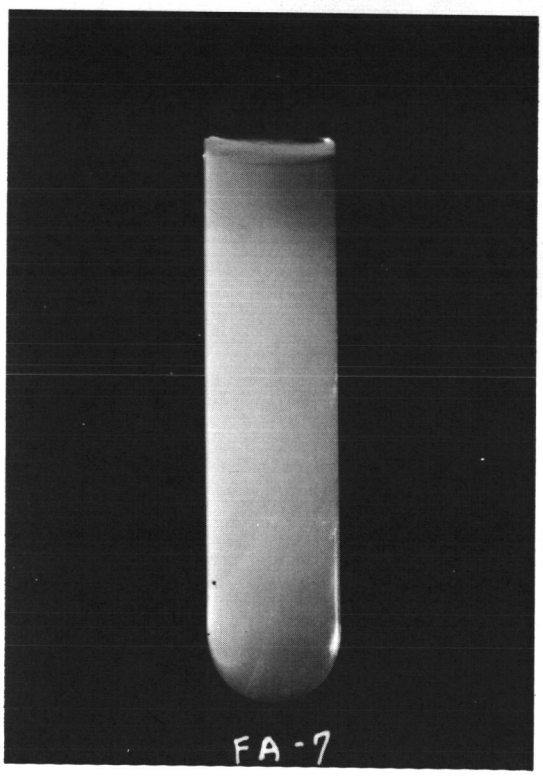


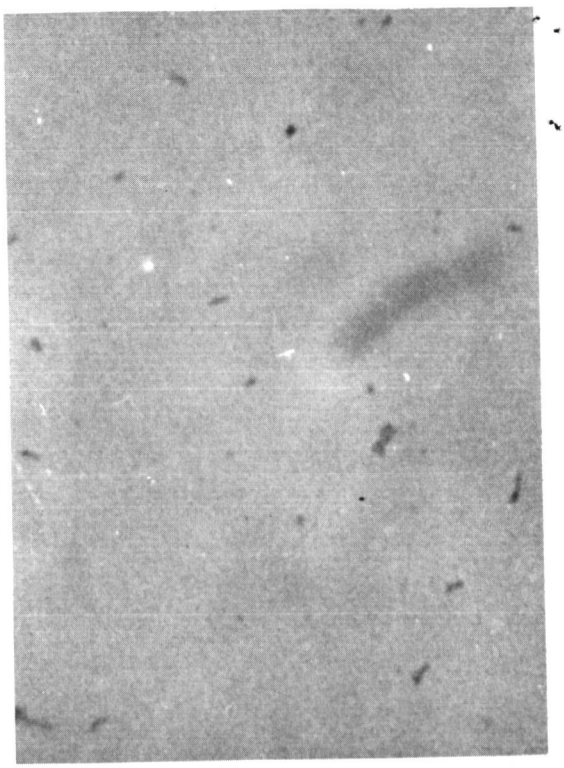
1 Week (X8000)



Agar Shake

Figure 4. Continued

24 Hours (X8000)



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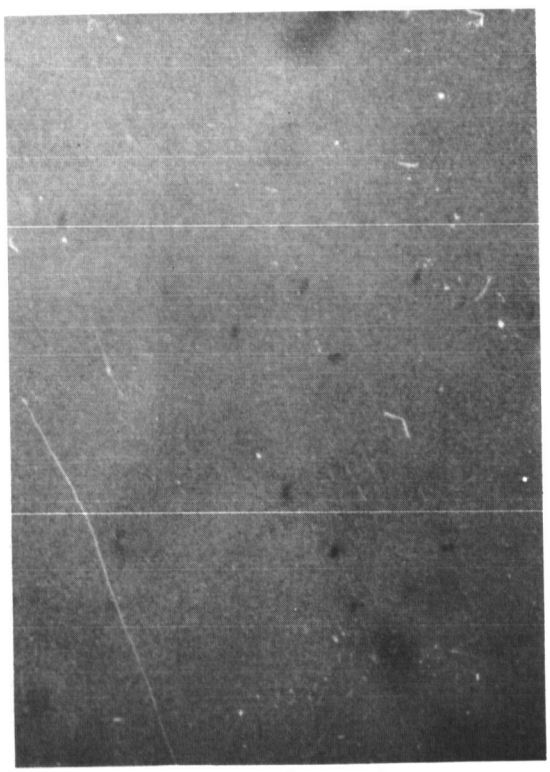

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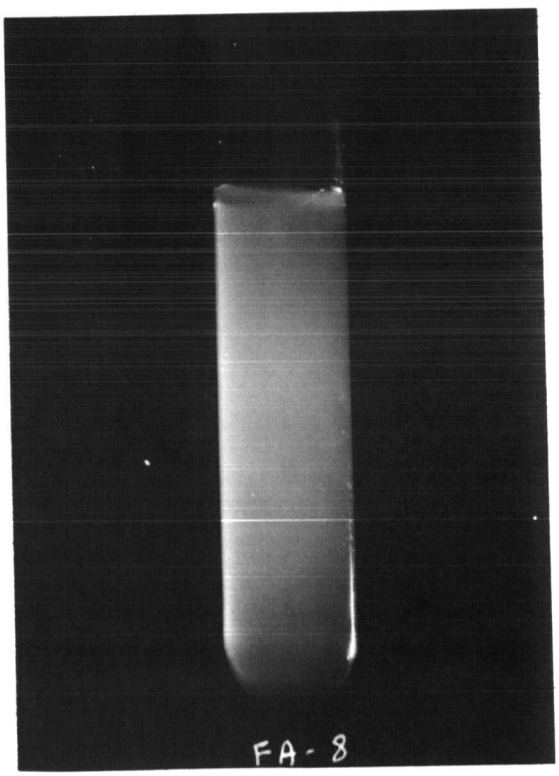
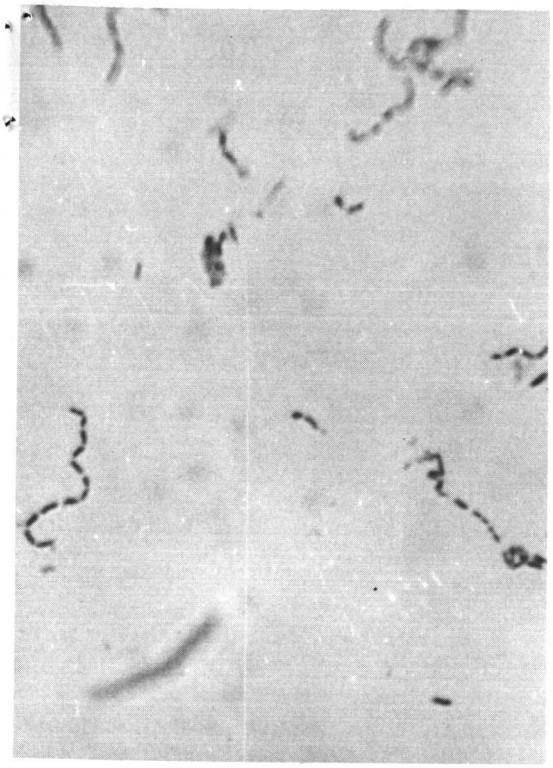


Agar Shake

Figure 4. Continued



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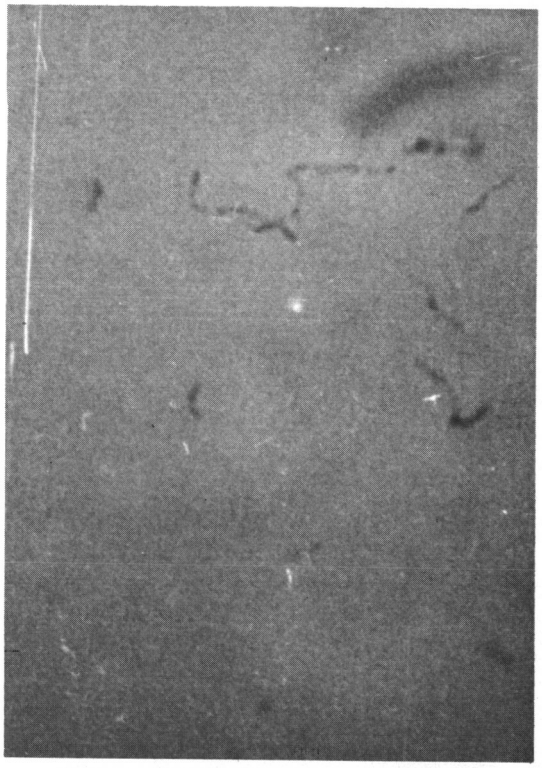

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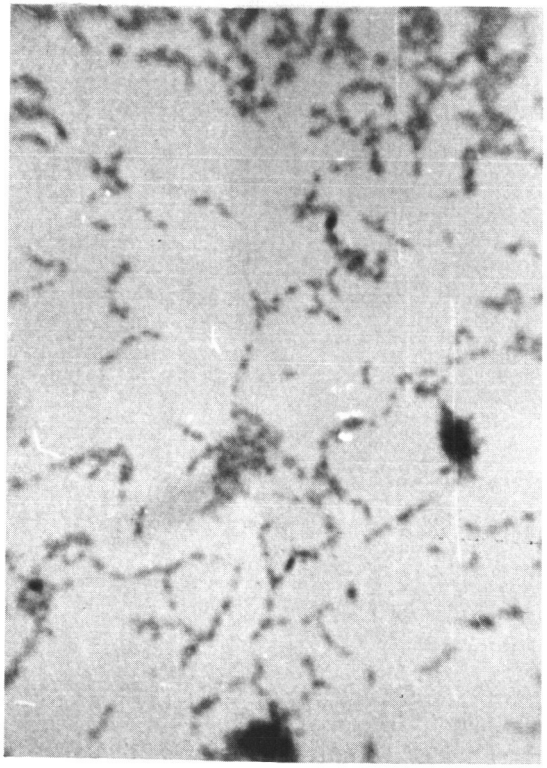

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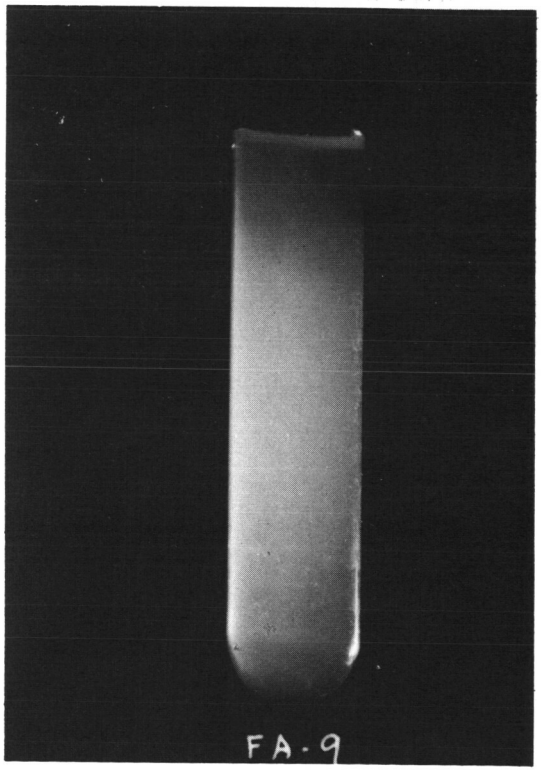
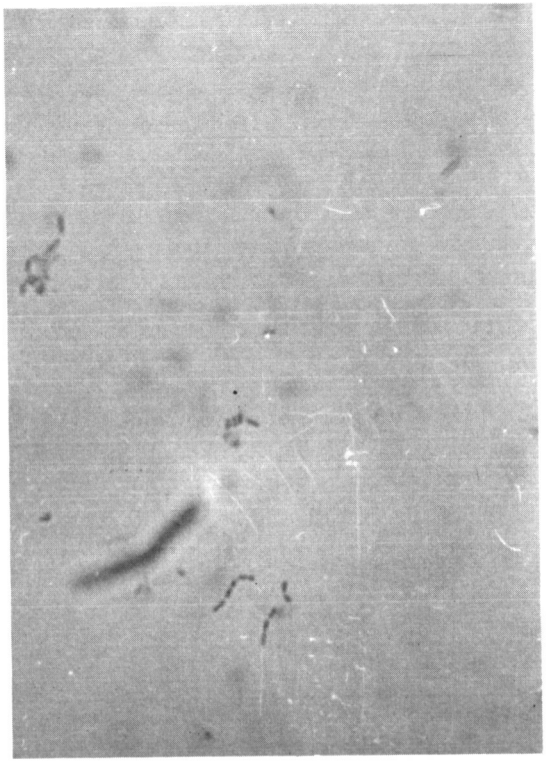


Agar Shake

Figure 4. Continued



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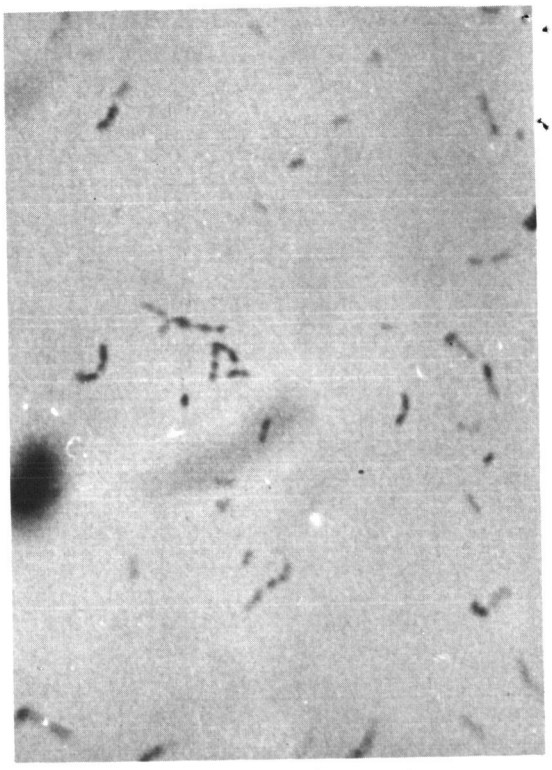

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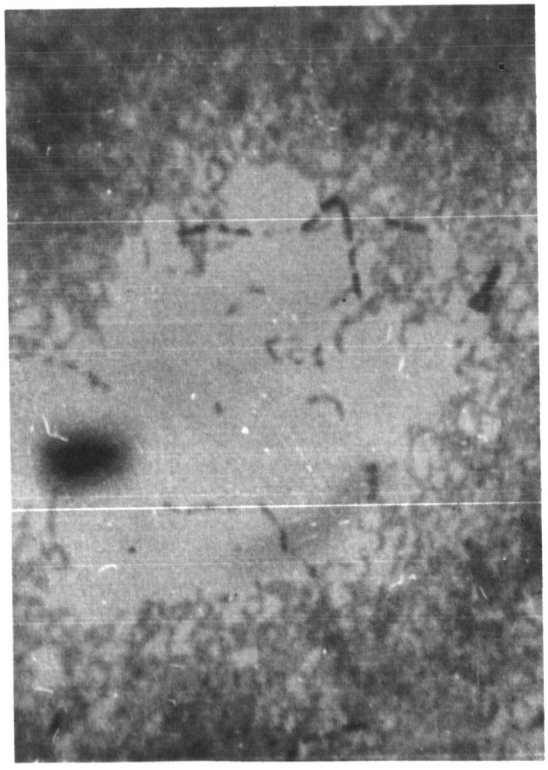
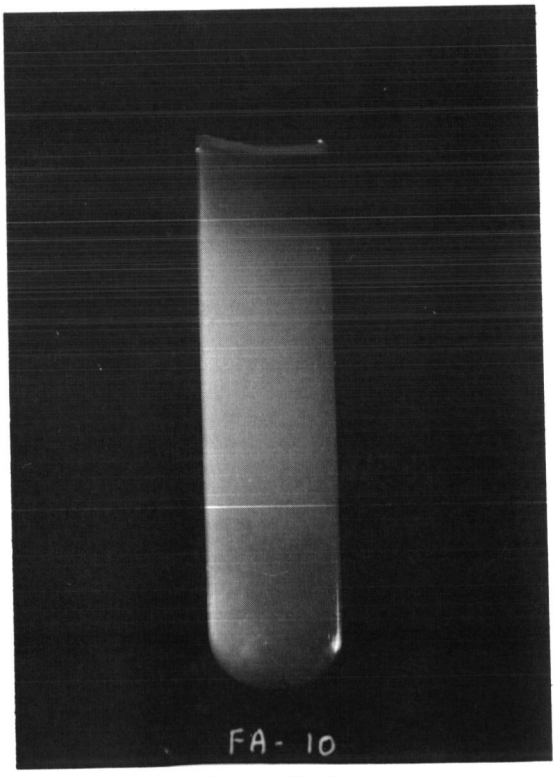
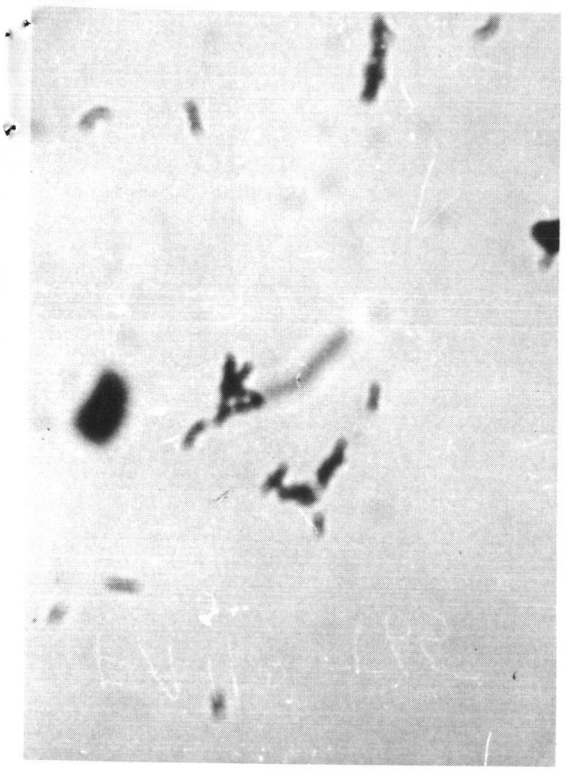


1 Week (X8000)

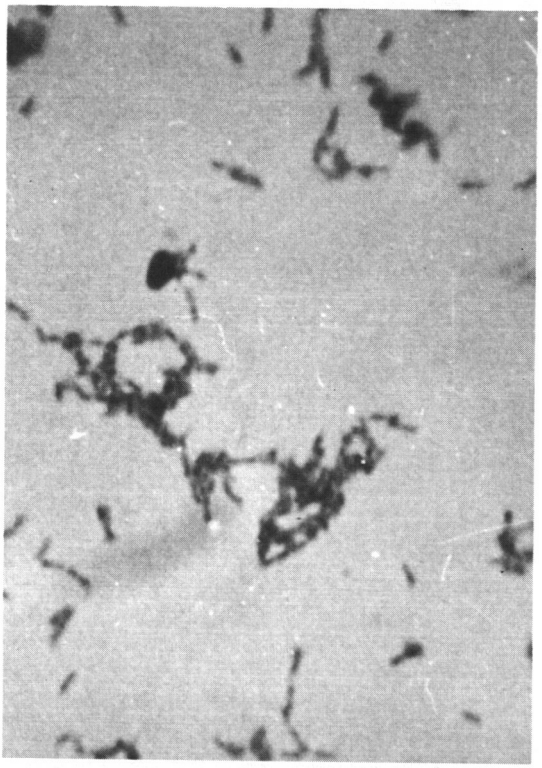


Agar Shake

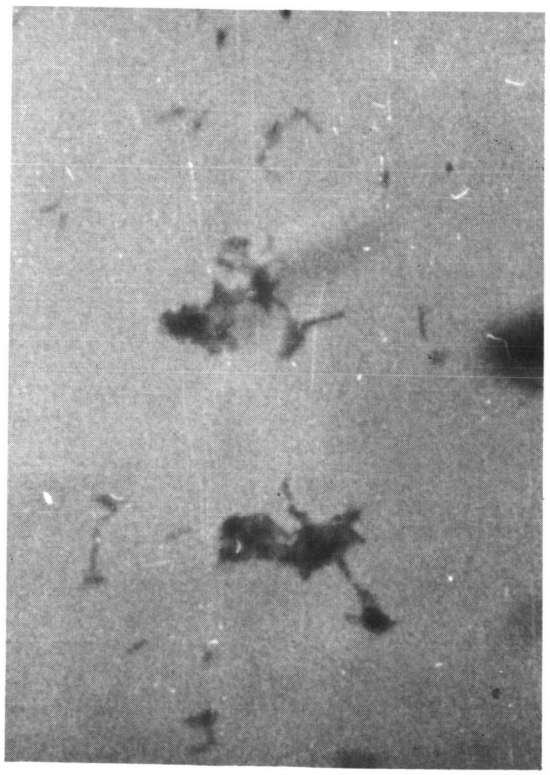
Figure 4. Continued



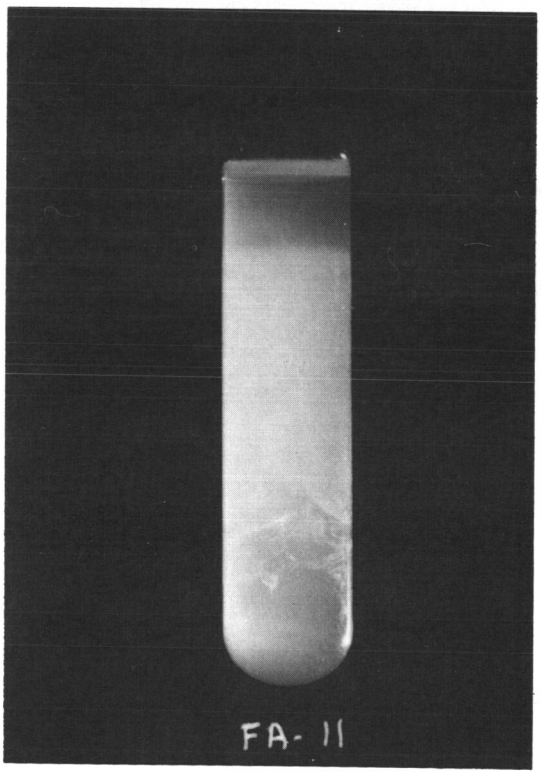
24 Hours (X8000)



48 Hours (X8000)

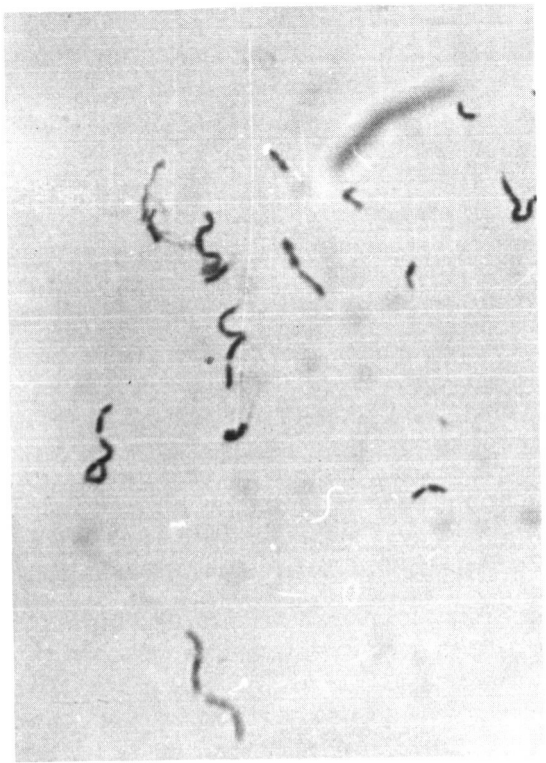


1 Week (X8000)



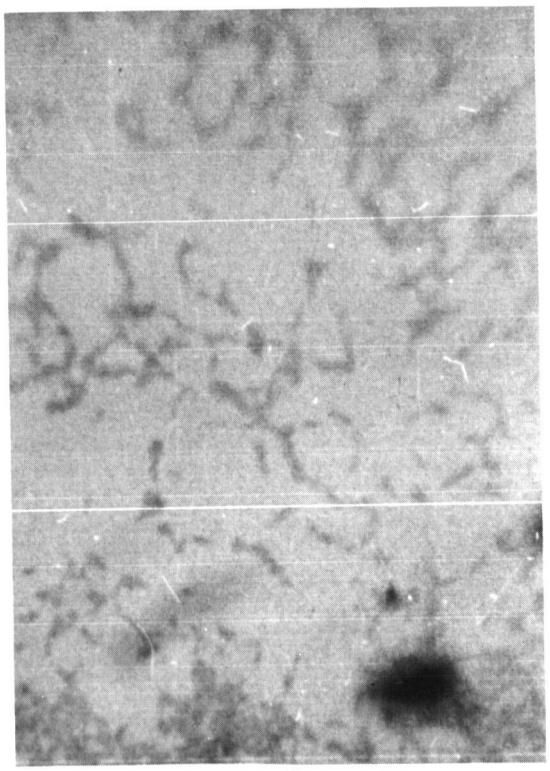
Agar Shake

Figure 4. Continued

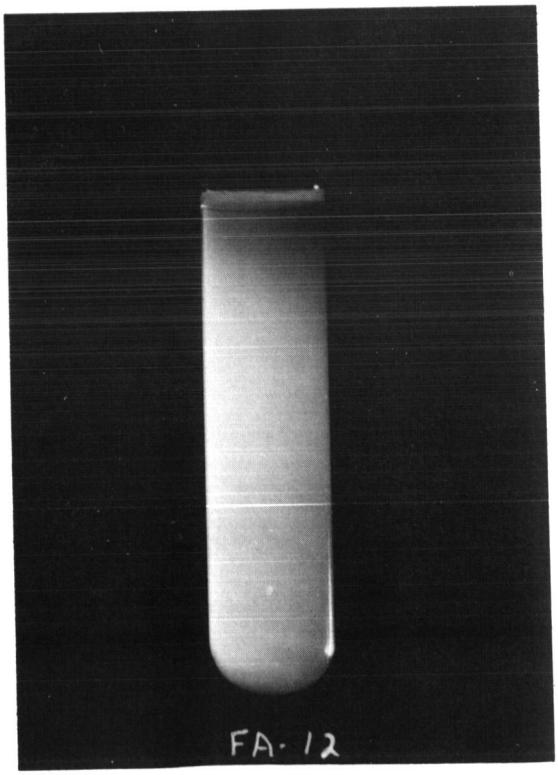


24 Hours (X8000)

48 Hours (X8000)

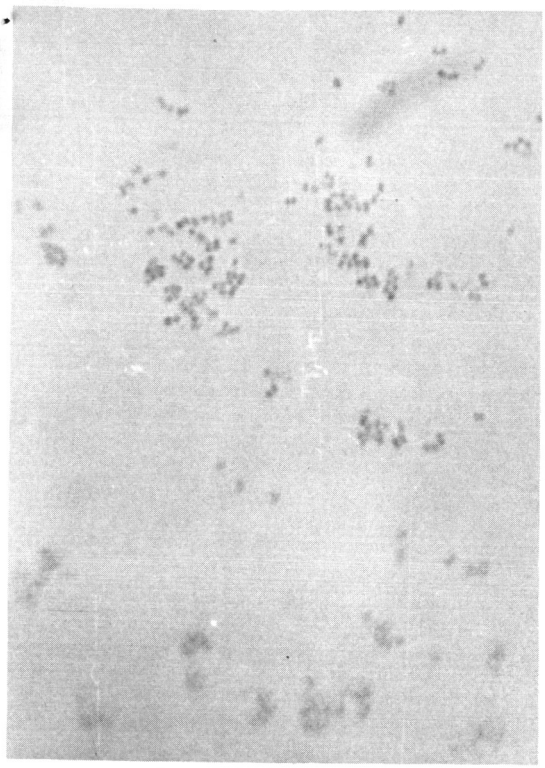


1 Week (X8000)

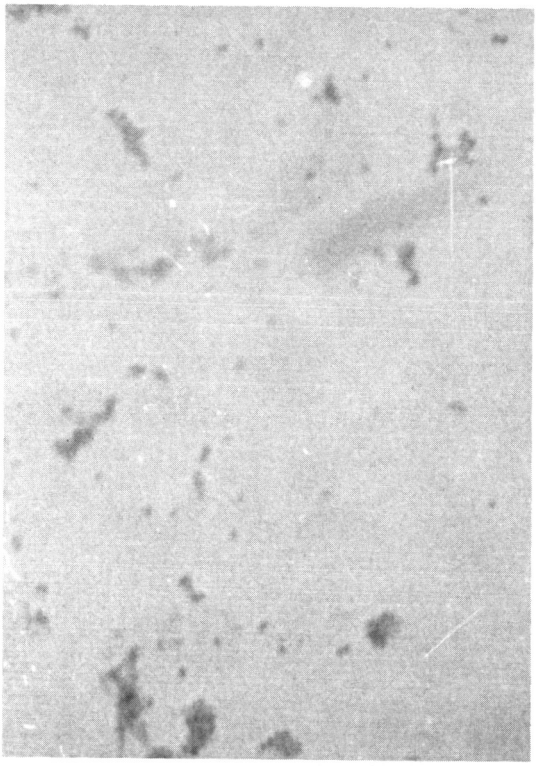


Agar Shake

Figure 4. Continued

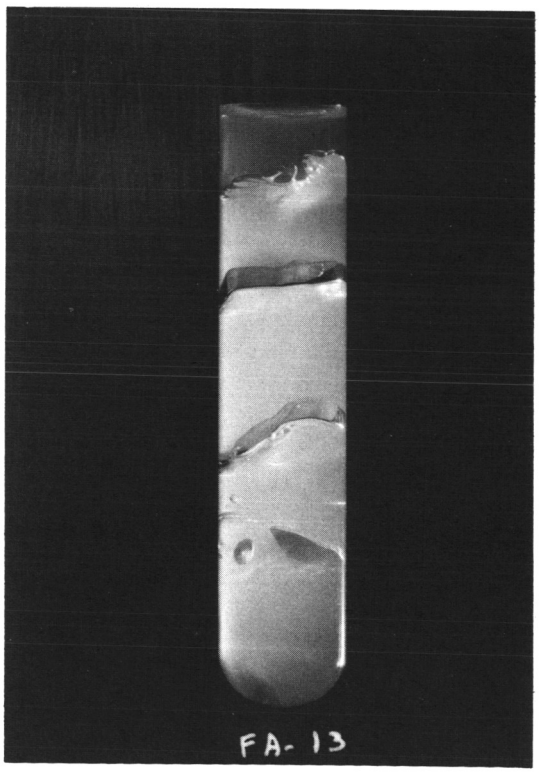


24 Hours (X8000)



48 Hours (X8000)

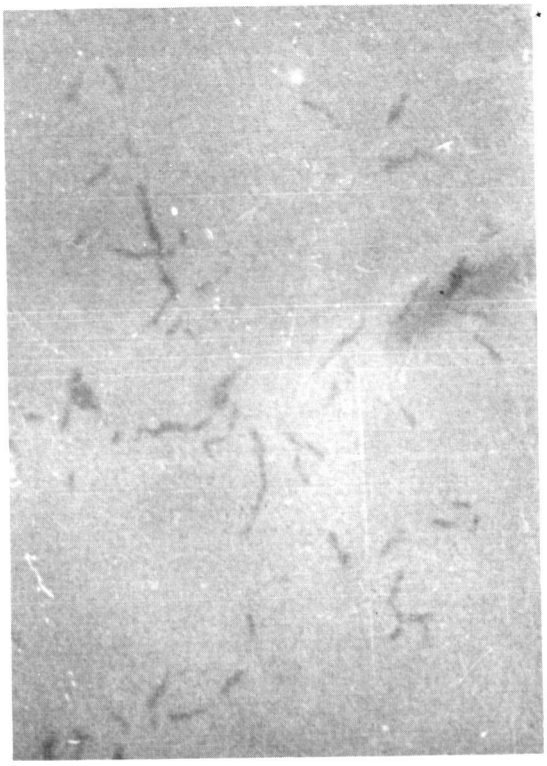
1 Week (X8000)



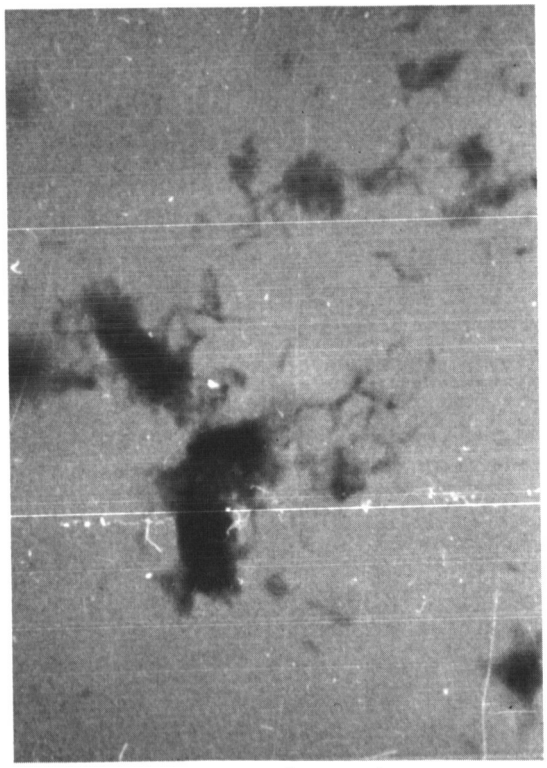
Agar Shake

Figure 4. Continued

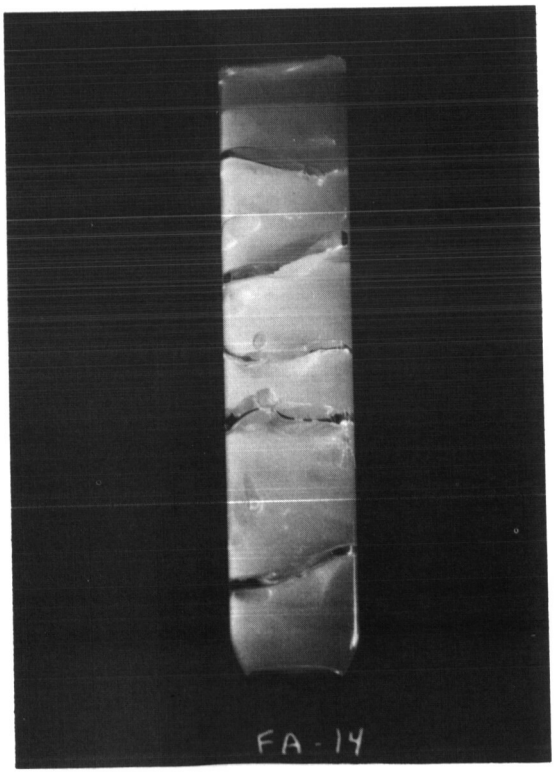
24 Hours (X8000)



48 Hours (X8000)

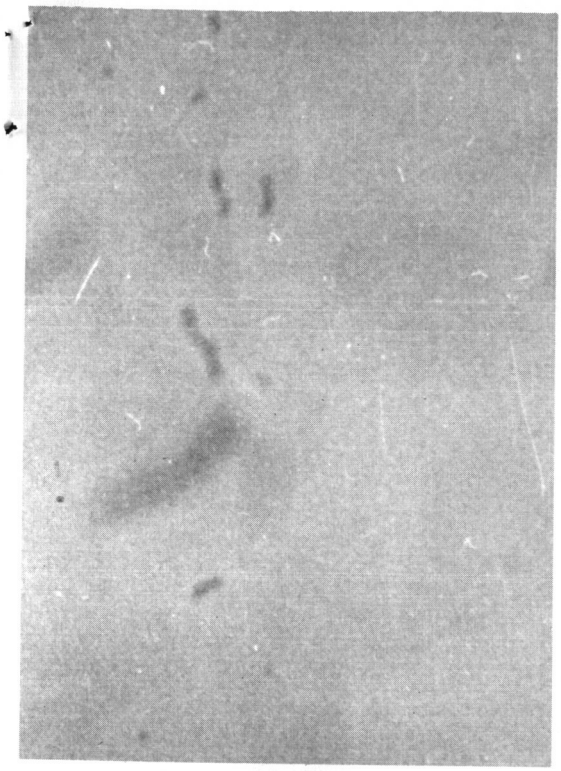


1 Week (X8000)



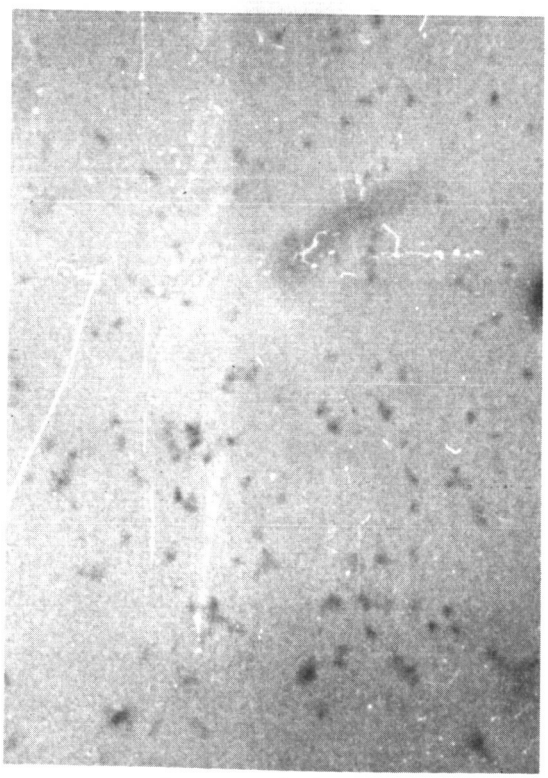
Agar Shake

Figure 4. Continued

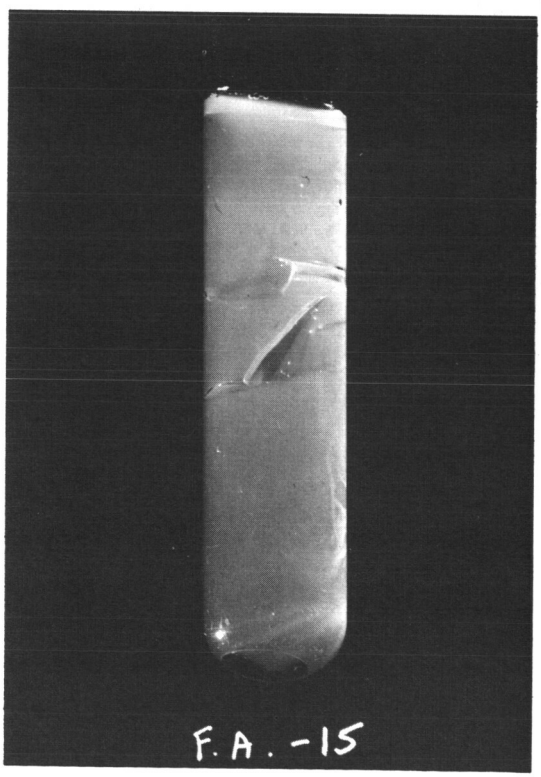


24 Hours (X8000)

48 Hours (X8000)

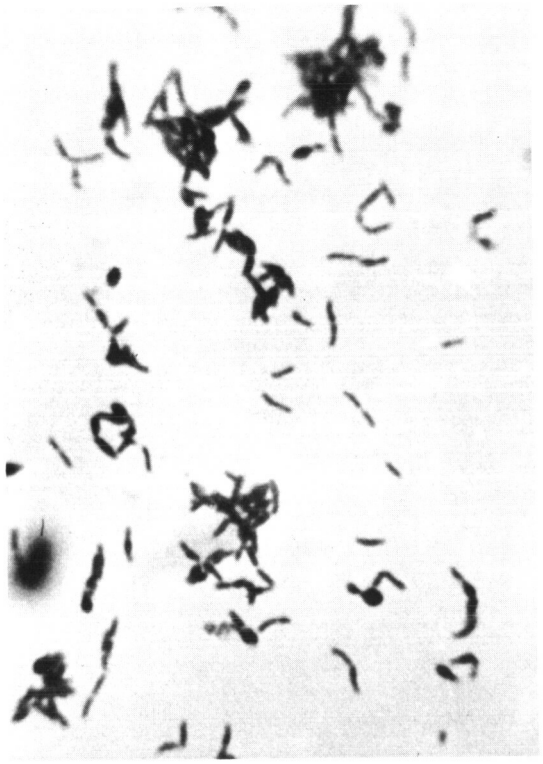


1 Week (X8000)



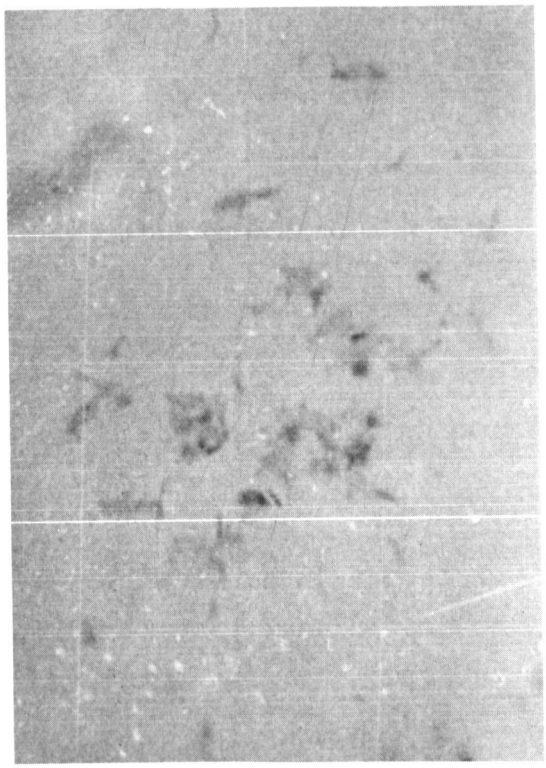
Agar Shake

Figure 4. Continued

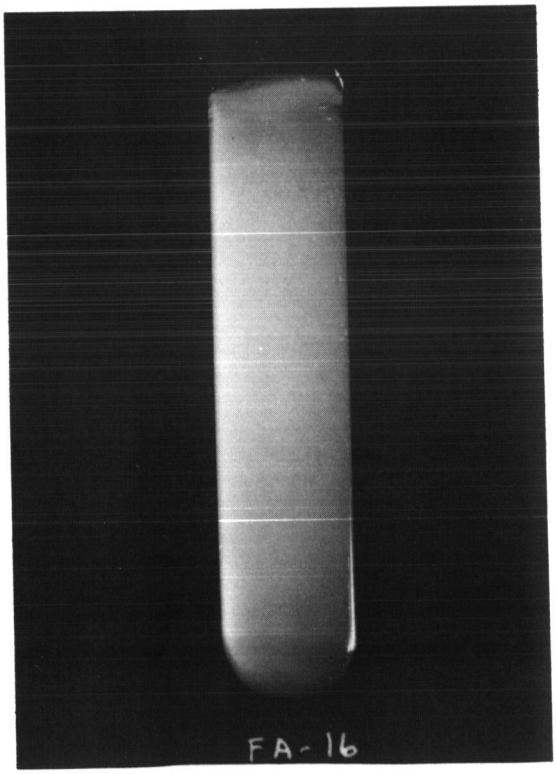


24 Hours (X8000)

48 Hours (X8000)

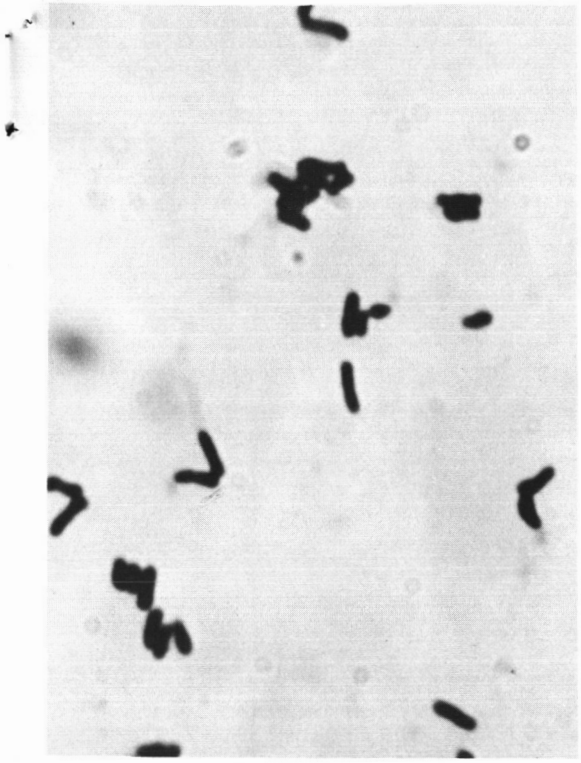


1 Week (X8000)



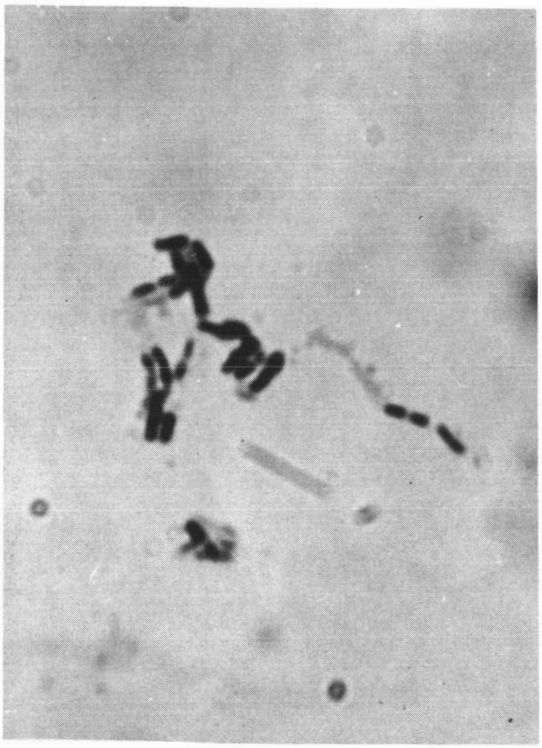
Agar Shake

Figure 4. Continued

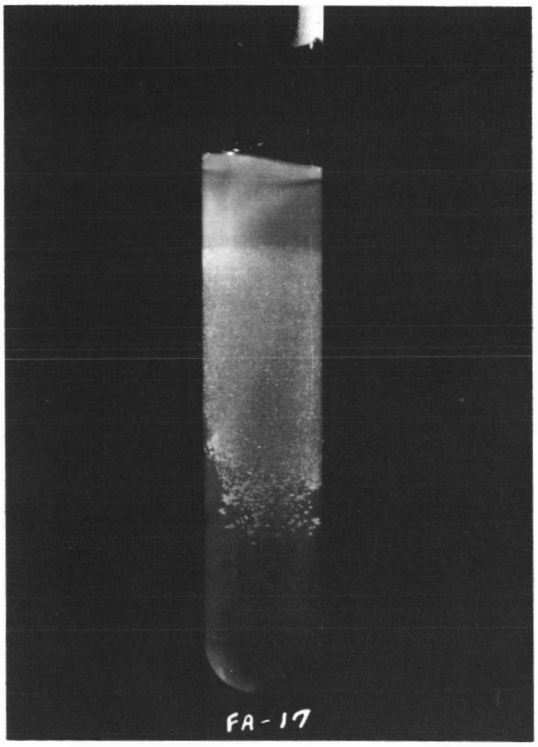


24 Hours (X1250, enlarged 8X)

48 hours (X1250, enlarged 8X)

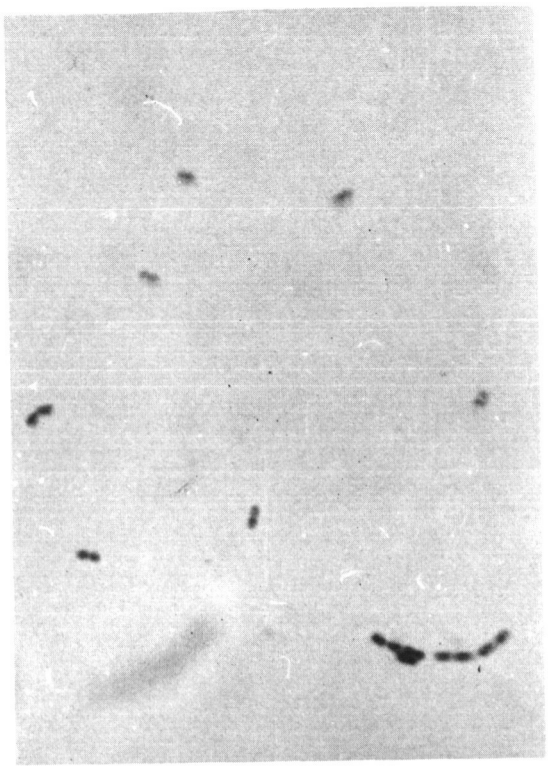


1 Week (X1250, enlarged 8X)



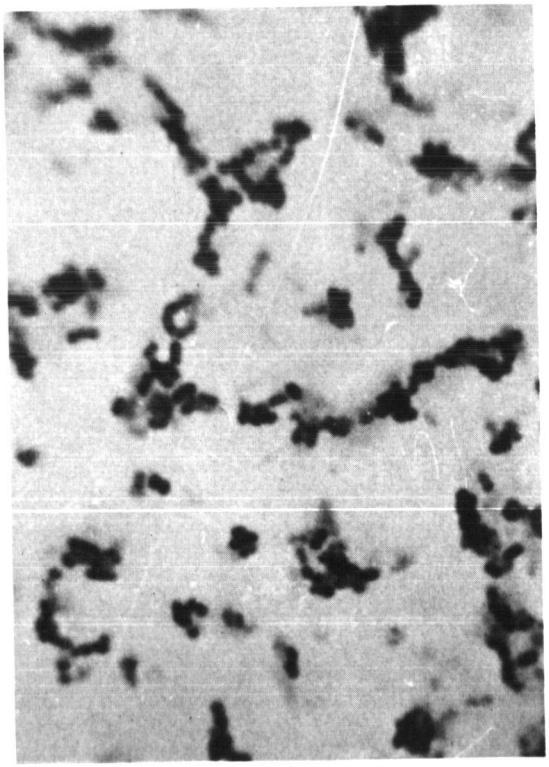
Agar Shake

Figure 4. Continued

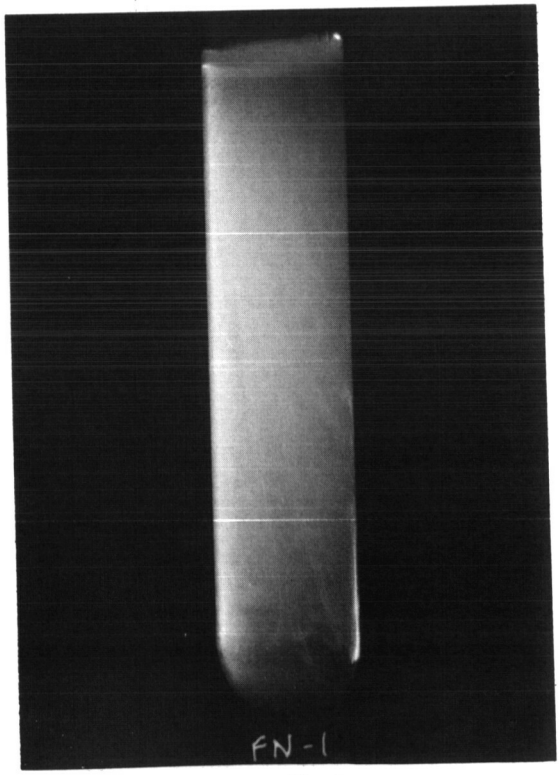


24 Hours (X8000)

48 Hours (X8000)

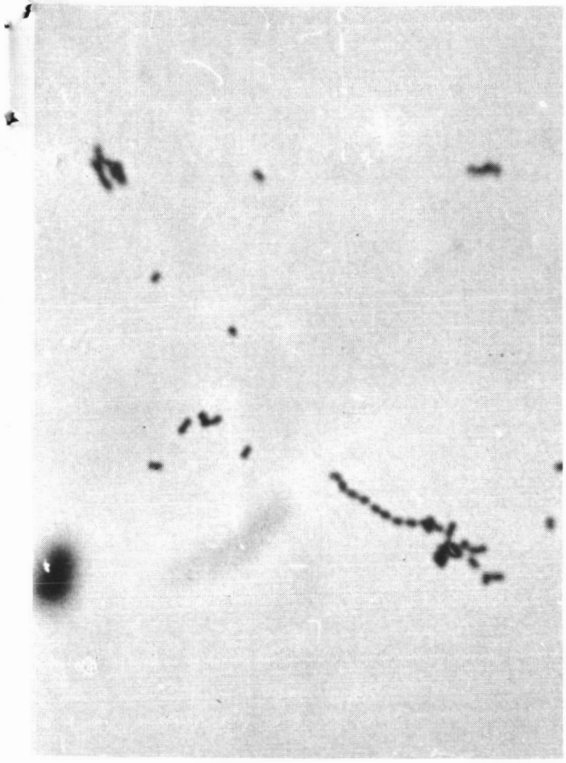


1 Week (X8000)

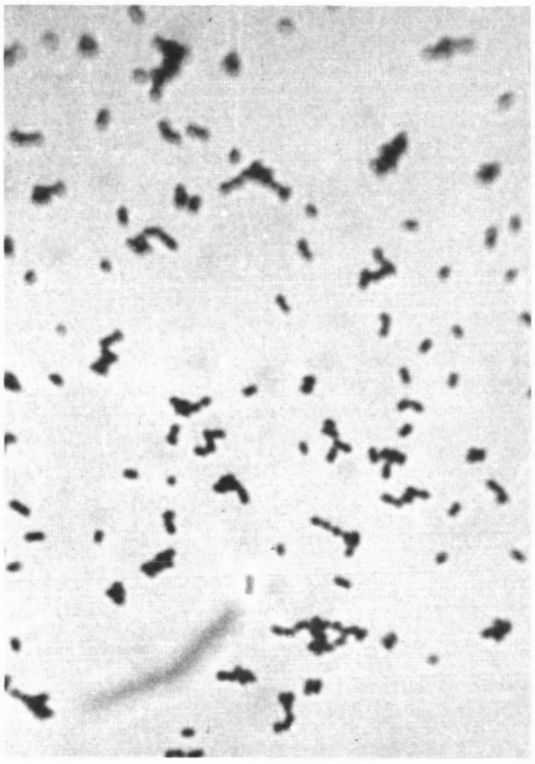


Agar Shake

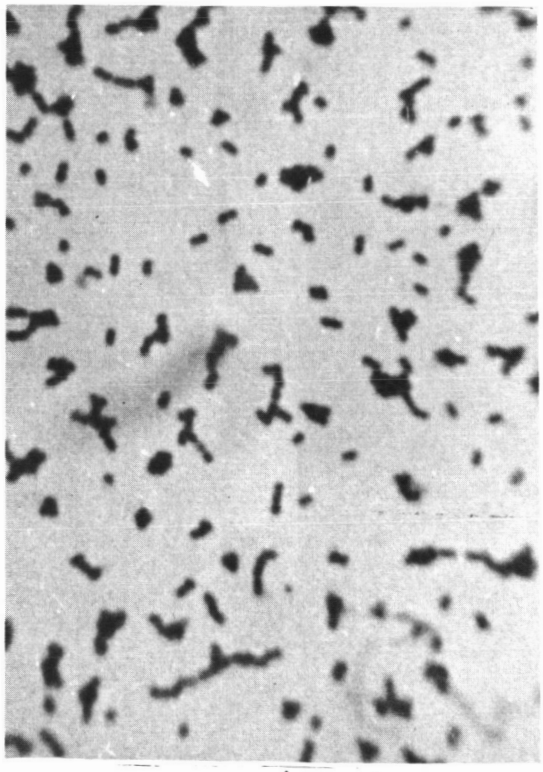
Figure 4. Continued



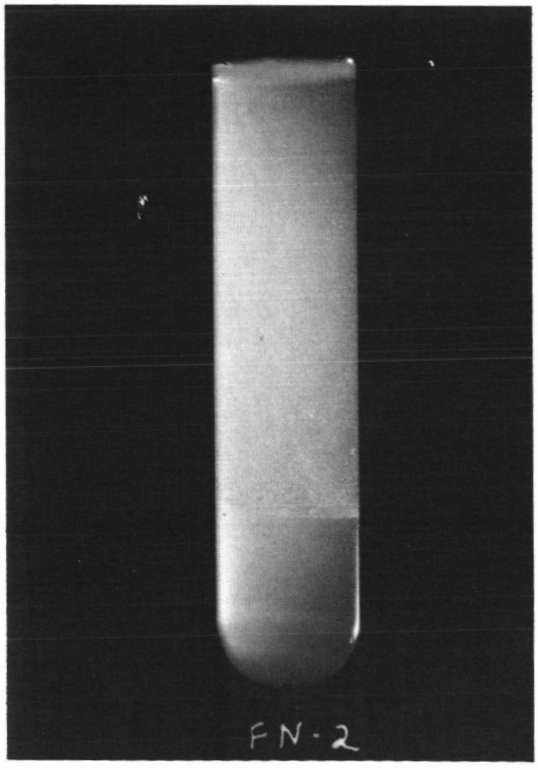
24 Hours (X8000)



48 Hours (X8000)

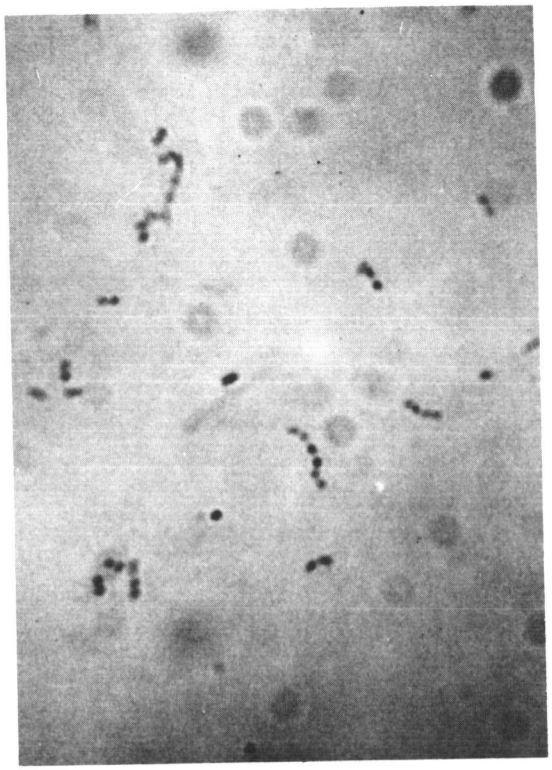


1 Week (X8000)

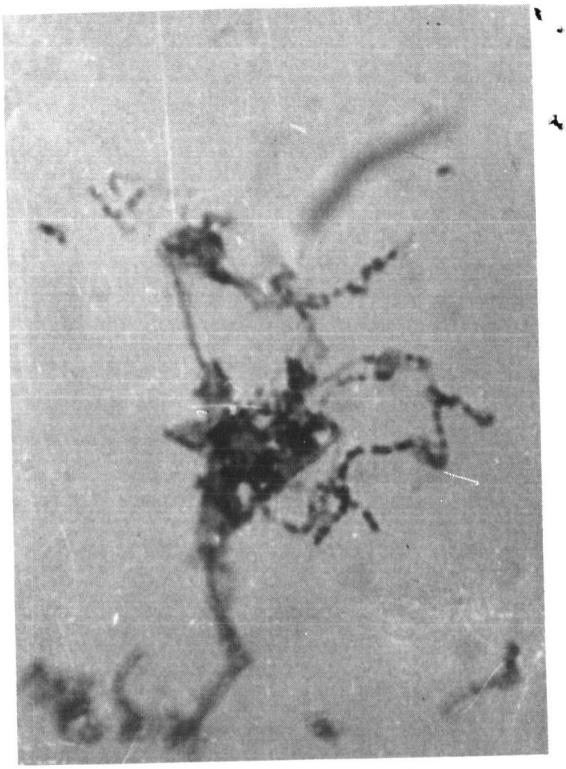


Agar Shake

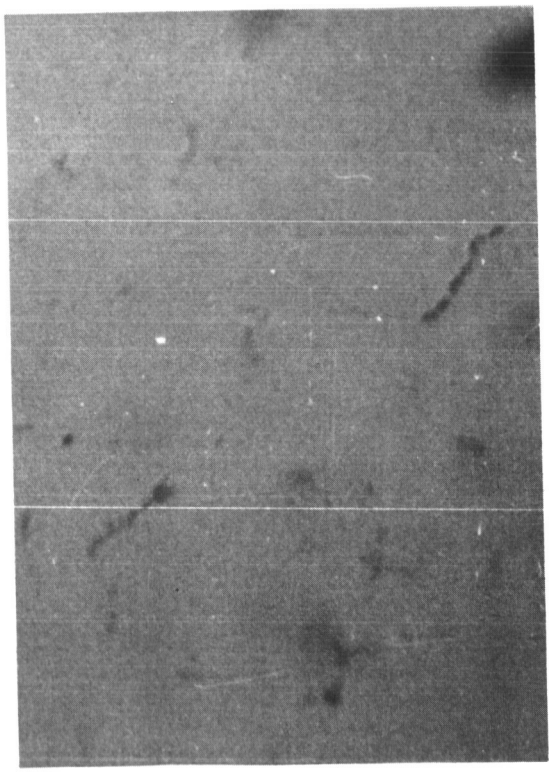
Figure 4. Continued



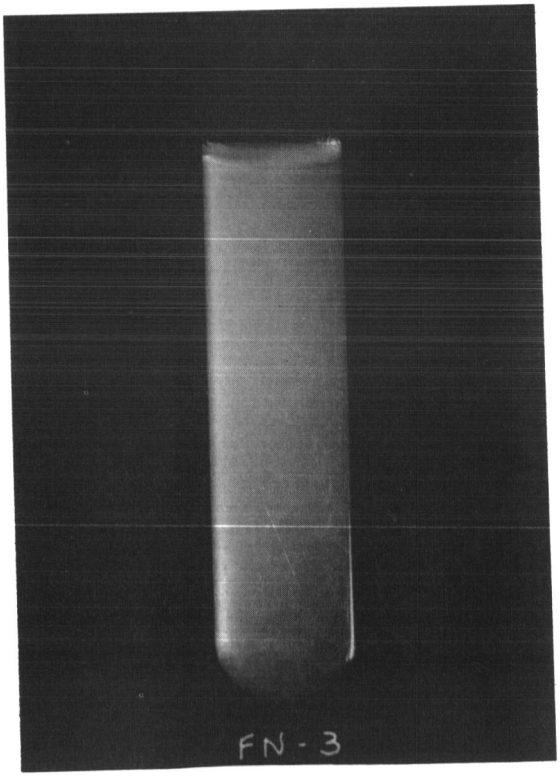
24 Hours (X8000)



48 Hours (X8000)

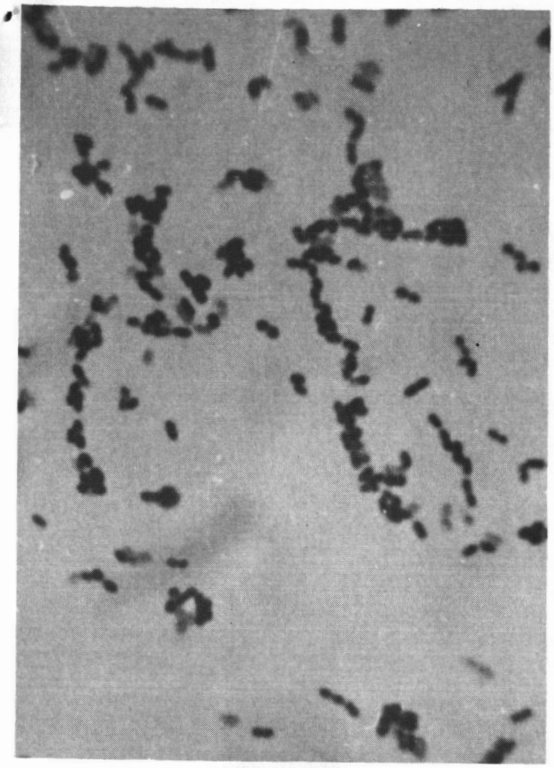


1 Week (X8000)

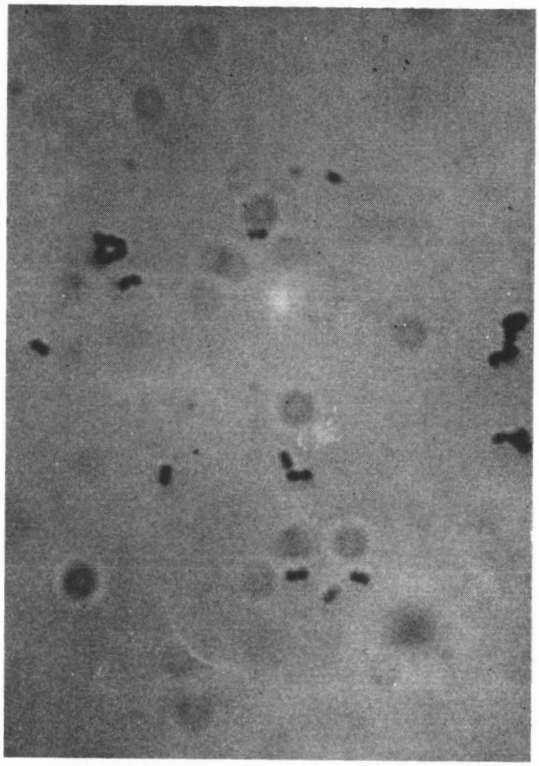


Agar Shake

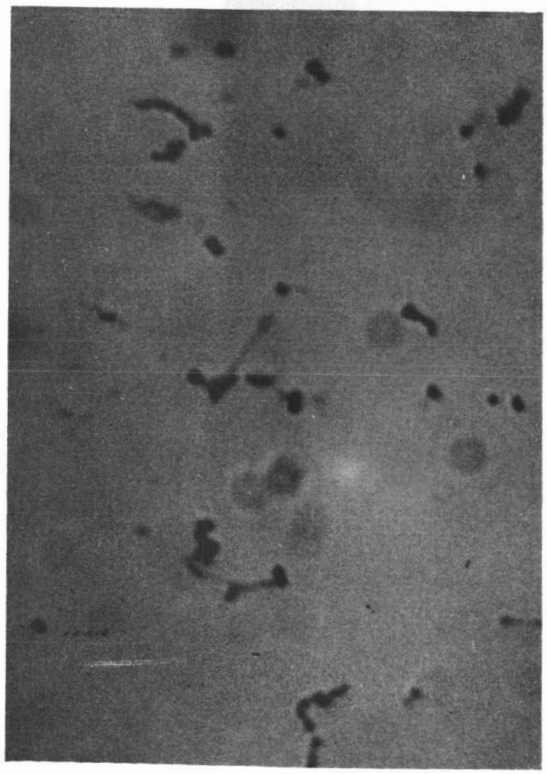
Figure 4. Continued



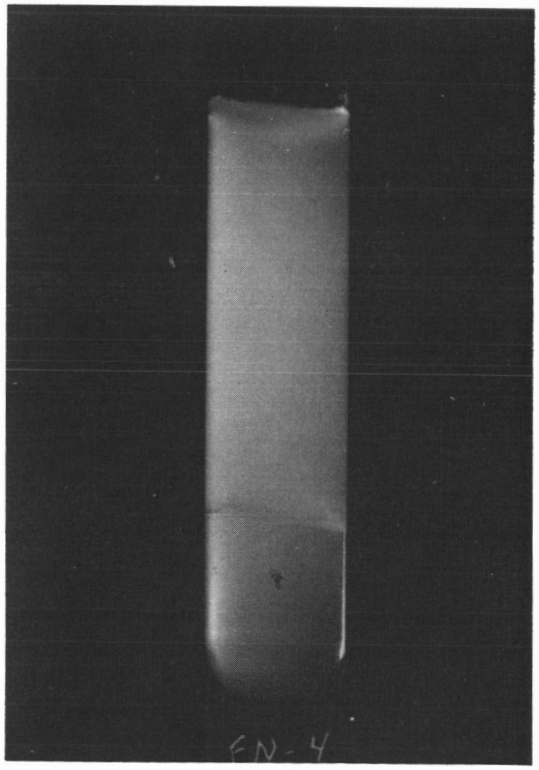
24 Hours (X8000)



48 Hours (X8000)



1 Week (X8000)



Agar Shake

Figure 4. Concluded

9/5/07

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—NATIONAL AERONAUTICS AND SPACE ACT OF 1958

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